CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-368

PHARMACOLOGY REVIEW

GENERAL PHARMACOLOGY/TOXICOLOGY COVER SHEET

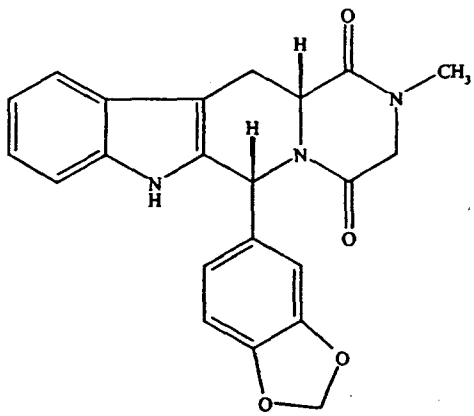
NDA No: 21-368 **Review No:** 1
Sequence No: 000 **Date/type of submission:** June 28, 2001/Original
Information to sponsor: Yes (x) No ()
Sponsor: Lilly ICOS LLC, Eli Lilly & Company, Indianapolis, IN 46285
Manufacturer for drug substance: Eli Lilly & Co., Tippecanoe Laboratories, Lafayette, IN 47909

Reviewer: Yangmee Shin, Ph.D.
Division: Division of Reproductive and Urologic Drug Products, HFD-580
Review completion date:

Drug:

Trade name: Cialis **Generic name:** Tadalafil
Code name: IC351 (LY450190)
Chemical name: Pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-, (6R-12aR)-

CAS registry No: 171596-29-5 **Mole file No:**
Molecular formula: $C_{22}H_{19}N_3O_4$ **Molecular weight:** 389.41
Structure:



Relevant INDs/NDAs/DMFs: IND 54,553 .
Drug class: β-carboline phosphodiesterase (PDE) type 5 inhibitor
Indication: Erectile Dysfunction (ED)

Clinical formulation: Yellow, film-coated, almond-shaped tablets containing 20 mg of tadalafil and inactive ingredients of lactose monohydrate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, iron oxide, croscarmellose sodium, sodium lauryl sulfate, microcrystalline cellulose, talc, titanium dioxide, triacetin & magnesium stearate.

Route of administration: Oral

Proposed clinical use: Treatment of erectile dysfunction

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Studies reviewed within this submission:

PHARMACOLOGY (#S21422, #01-0007-11, #00-0010-11, #00-0009-11)
TOXICOKINETICS (#D01899, # — 88780, # — 88779, # —-353016)
TOXICOLOGY
1-Year oral toxicity study in beagle dogs (vol. 31, p 1, #D01899)
CARCINOGENICITY
2-Year oral carcinogenicity study in CD-1 mice (vol. 33, p 1, # — 88455)
2-Year oral carcinogenicity study in l— Wistar rats (vol. 34, p 1, # — 88203)
REPRODUCTIVE TOXICOLOGY
Segment II & III reproductive study in CD rats (vol. 37/38, p 1, # — -353010,—-353016)

Studies <u>not</u> reviewed within this submission (Appendix I):

<u>IND 54,553 Review #1, May 26, 1998</u>
PHARMACOLOGY (#97-001-14, #97002-14, #97-003-14, # — 94-007)
SAFETY PHARMACOLOGY (#20215, #S20996, #21011, #S20222, #97-004-14)
PHARMACOKINETICS
Absorption (#R21147, #D21148, #BPW662, #BPW641/BPW659)
Metabolism (#BPW549/BPW564, #BPW641/BPW659)
Distribution (#BPW618)
Protein binding (#BPW507, #BPW495)
TOXICOKINETICS (#R20861, #R21236, #D21148, #D20786, #D20863, #D21235)
TOXICOLOGY
Acute toxicity (#M20798, #M20799, #M20977, #M20978, #R20796, #R20797, #R20979, #R20980)
Repeated toxicity
1. Maximum repeatable daily oral dosage study in the — Wistar Rat (#R20791)
2. 1-Month oral toxicity study in — Wistar Rats (#R20861)
3. Study to determine the maximum repeatable daily oral dosage in the beagle dog (#D20786)
4. 1-Month oral toxicity study in the beagle dog (#D20863)
5. 6-Month oral toxicity study in the beagle dog (#D21235)

GENETIC TOXICOLOGY
1. Microbial mutagenicity study (#U20206)
2. Mouse lymphoma thymidine kinase mammalian cell mutation study (#V21166)
3. *In vitro* cytogenetic evaluation in cultured human lymphocytes (#V20918)
4. WHO nitrosation assay (#U21004)

<u>IND 54,553 Review #2</u>
TOXICOLOGY
1. 6-Month oral toxicity study in the beagle dog (#D21235)

<u>IND — Review #1, May 27, 1999</u>
PHARMACOKINETICS
Metabolism (#1999IV-RSL05, #006R00)
Excretion (#BPW549/BPW564)
TOXICOKINETICS (#88270)
TOXICOLOGY
1. 3-Month oral pilling toxicity study with a 13-week recovery period in the Beagle dog (#88270)

GENETIC TOXICOLOGY
1. Micronucleus assay in bone marrow of male — Wistar Rats (#R20937)

<u>IND — Review #2, Jul 26, 1999</u>
SAFETY PHARMACOLOGY (#PG9927)
TOXICOKINETICS (#M04298, #R18498, #M04398, # — -353004, # — -353005)
TOXICOLOGY
1. 1- & 3-Month oral gavage toxicity in CD-1 mice (#M04298)

REPRODUCTIVE TOXICOLOGY
1. Embryo/fetal development in CD-1 mice (# —353004)
2. Embryo/fetal development in CD rats (# —353005)

IND 54,553 Review #3, Aug 10, 1999
PHARMACOLOGY (#98-0001-11, #98-0002-11, #98-0003-11)
PHARMACOKINETICS (#132R98, #R14998, #M04198, ADME#6, ADME#7)
TOXICOKINETICS (# 88440, #21236)
TOXICOLOGY
1. 3-Month oral gavage toxicity in CD-1 mice (#88437)
2. 6-Month oral gavage toxicity in — Wistar rats (#21236)

REPRODUCTIVE TOXICOLOGY
1. Oral gavage fertility study in CD rats (#96364)

JUSTIFICATION FOR 2-YEAR CARCINOGENICITY STUDY DOSE SELECTIONS IN RATS & MICE

IND — Review #3, Sep 3, 1999
PHARMACOLOGY (#PR9902)
PHARMACOKINETICS (#B00199, #R18498)
TOXICOKINETICS (#R18498, — 88632)
TOXICOLOGY
1. 3-Month oral gavage in Fisher 344 rats (#R18498)
2. 6-Month oral pilling toxicity study with a 3-month recovery period in the Beagle dog (88632)

SPECIAL TOXICOLOGY
1. *In vitro* ocular irritation-agar diffusion cytolysis & aqueous pH in cultured rabbit cornea cells (#990416ADC)

IND — Review #4, Dec 27, 1999
PHARMACOLOGY (#PR9902)
SPECIAL TOXICOLOGY
1. *In vivo* eye irritation study in New Zealand White rabbits (#SLI3130.495)
2. *In vivo* acute dermal toxicity in New Zealand White rabbits (#SLI3130.487)

IND — Review #5, Aug 15, 2000
PHARMACOLOGY (#1999IV-EI004, #PR9906, #99-0005-11)
PHARMACOKINETICS
Distribution (#003R00)
Metabolism (#1999IV-SF038)
Excretion (#078R99, #002R00)
Repeat Dose in Monkeys (88548)

Introduction and drug history: IC351 is a potent, competitive and reversible inhibitor of cGMP specific PDE type 5 for an indication of ED under IND 54,553. It is also currently being investigated for — under IND — Major toxicities are irreversible seminiferous testicular atrophy and vasculitis in dogs. The original submission by ICOS Corporation was placed on clinical hold because of vasculitis findings in dogs and the high daily clinical dose up to 100 mg.

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on Approvability: The preclinical studies conducted support the safety of the proposed dose of 20 mg of Cialis.

B. Recommendation for Nonclinical Studies: The 2-year carcinogenicity studies in male rats, and male and female mice were conducted at doses below those recommended by the ICH guidelines (see Exectutive CAC minutes in appendex II) based on the AUC exposures for the 20 mg human dose. The Committee recommended an additional alternative mouse carcinogenicity assay be conducted for Phase IV commitment unless the sponsor provided evidence for saturation of absorption by measuring either total radioactivity or metabolites.

C. Recommendations on Labeling: Refer to the labeling comments.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Effective antihypertensive oral doses of IC351 were 1 mg/kg in the rat. Reduction in mean blood pressure occurred at doses from 20 mg/kg without effects on heart/respiration rate in conscious dogs, but moderate tachycardia was seen in a dog (1/2) at 30 mg/kg and in both dogs at 100 mg/kg in another study. The cardiovascular effects were not observed in the repeated toxicity studies. IC351 potentiated atrial natriuretic factor (ANF)-induced diuresis and natriuresis in rats at lower doses (0.1 mg/kg, i.v.) than those required for decreasing blood pressure. Slight-to-moderate ptosis and depression of the pinnal reflex were observed in rats given 200 mg/kg. IC351 did not cause death up to 2,000 mg/kg (p.o.) in mice and rats in acute studies. Like other PDE5 inhibitors, the major findings of IC351 treatment in repeated dose studies are arteritis and testicular degeneration/atrophy observed in multiple species. IC351 was not genotoxic and the carcinogenicity studies were negative although hepatocellular adenomas/carcinomas were observed with increased frequency in high dose male mice and rats. Reproductive and developmental studies in mice and rats displayed no adverse effects on fertility at doses up to 1,000 mg/kg. Mice were used for a second rodent species of embryo/fetal development studies since plasma exposure for rabbits was minimal. A NOAEL for maternal toxicity was established at 1,000 mg/kg in mice and 200 mg/kg in rats (based on reduced body weight gain). A NOAEL for F1 developmental toxicity in the rat could not be identified due to significantly reduced postnatal survival in all dose groups from the combined segment II/III study. Sponsor defined a NOAEL of 30 mg/kg from a subsequent study, which gives 9-fold exposure for the unbound parent drug (pregnant rat) to the human exposure at 20 mg. IC351 is a mild ocular and dermal irritant in New Zealand White rabbits.

B. Pharmacologic Activity: IC351 is a potent and selective inhibitor of PDE5 among the PDEs tested *in vitro*. PDE5 is a major cGMP-hydrolyzing enzyme in human cavernosal smooth muscle, and the inhibition of PDE5 by IC351 enhances relaxant effects of NO by stimulating cGMP levels. This leads to relaxation of penile resistance arteries and the smooth muscle to enhance the erectile response. IC351 strongly potentiated the inhibitory effects of sodium nitroprusside (SNP) on human platelet aggregation with complete inhibition at 0.25 μM, and on increased cGMP levels in human cavernosal smooth muscle, suggesting that the PDE5 inhibition by IC351 may lead to large increases in cGMP levels once activated. IC351 retains relatively low selectivity for PDE5 vs. human PDE11A (abstract from Am. Coll. Clin. Pharmacol., VA, 2001), which was widely expressed in kidney, liver, pituitary/salivary glands and testis (PNAS 97: 3702, 2000). Thus, pharmacological characterization of IC351 on human PDE11A may provide additional information on the mechanism of IC351.

C. Nonclinical Safety Issues Relevant to Clinical Use:

1. Testicular degeneration/atrophy were observed with increased incidence in the 3-month toxicity study and the carconogenicity study in mice and in the 3-, 6- and 12-month toxicity studies in dogs with no/low safety margin at a NOAEL compared to the proposed human dose of 20 mg. The findings are likely to be irreversible since the incidence was observed during the recovery in the 3- and 6-month dog studies. In men, there were no clinically significant effects on semen parameters up to 6 months with a clinical dose of 20 mg (#H6D-MC-LVCZ).

2. Vasculitis findings should be interpreted cautiously since the relevance to humans and the pathogenic mechanism of drug-related vascular lesions in animals are poorly understood, and the specific biomarkers are not identified. IC351 treatment increased the incidence of vasculitis in mice, rats and dogs but the effects varied considerably between studies. In a 13-week study in mice, there was hemorrhage in mesenteric lymph nodes in the high dose group (400 mg/kg, approximately 9 times the maximum human exposure of unbound drug). In another 3-month study, there was minimal vasculitis in the high dose group receiving 800 mg/kg (7 times the maximum human exposure; exposure estimates varied between and within studies). In Wistar rats, vasculitis occurred in a number of tissues with slight/minimal severity. In general, the incidence was only slightly higher in treated groups than in controls. These effects were seen at exposures anywhere from 2X (mesenteric phlebitis) to 33X the maximum human exposure. In dogs, findings of arteritis occurred in 1 and 6 month studies. Effects included perivascular inflammation in the lungs, increased incidence of coronary arterial lesions and marked disseminated arteritis. These findings occurred in the absence of elevated heart rate and resulted in the drug being placed on clinical hold. The study pathologist, ——— concluded "the high incidence of arteritis that has been associated with high doses of IC351 in the 6-month dog study, and the predominance of arterial changes in the mid- and high-dose groups in the 1-month study are strongly suggestive of a treatment related change or treatment related exacerbation of the spontaneous polyarteritis". In general, drug effects were seen at exposures between 5 and 16 fold in the 1-month study, and between 29 and 54 times in the 6-month study (as measured by mean AUC of unbound drug) the maximum human exposure. Due to the concerns about vasculitis, the Division requested a 12-month toxicology study in dogs. This study was essentially negative at exposures of 3-33 times the maximum human exposure but there was marked neutropenia/thrombocytopenia indicative of type III immunopathy in two dogs (exposures 14 and 18X human exposure). The high dose dog did have perivasculitis in the circumflex branch of the left coronary artery with clinical signs of fever, anorexia and lethargy. In humans, symptoms of hypersensitivity such as myalgia, infection and back pain were the most frequently reported adverse events associated with IC351. The sponsor concluded that neither back pain nor myalgia was associated with inflammatory or myopathic etiologies based on a clinical study, which measured erythrocyte sedimentation rate and serum creatinine kinase.

In dogs, direct measurements of arterial diameter, vascular resistance or blood flow were not conducted to determine if exaggerated hemodynamic effects were associated with the vasculitis (clinical data indicate that combined treatment with antihypertensive drugs generally reduced the episode of myalgia and backpain). In the safety pharmacology studies, moderate tachycardia (40-60 bpm) was observed in dogs at doses ≥30 mg/kg (1/2 at 30 mg/kg & 2/2 at 100 mg/kg). The effective antihypertensive dose in rats is 1 mg/kg, po. In conscious dogs, single oral doses of 20 and 200 mg/kg produced slight reductions in mean blood pressure without effects on heart rate or respiration rate. In the 1-month study (D20863), the high dose produced a moderate decrease in heart rate with some vasculitis in the lung, spinal cord and thymus. In a 6-month study (D21235), there were no drug-related changes in ECG but there was increased incidence and severity of arteritis with clinical signs in the high dose dogs. It seems that IC351 can induce vascular changes in the absence of any significant effect on cardiac function.

In dogs with drug blood levels below approximately 29 times the human exposure, vascular effects, if any, were minimal to slight. In one 28-day study, D20863, there were positive effects (perivascular inflammation in the lungs in 3/6 dogs vs. 0/6 in controls) at approximately the same exposure as men taking 20 mg. In the 12-month study, exposures at the high dose were up to 33 times the human exposure to free drug with essentially negative effects. It is not known if humans are more or less sensitive to this effect than animals.

Although the data are not particularly convincing that the finding in animals is relevant to humans, hypersensitivity is the major manifestation of clinical drug-induced vasculitis. There were two dogs with symptoms of marked thrombocytopenia (14 and 18X the human exposure) indicative of type III immunopathy in the 12-month dog study and symptoms of back pain and myalgia as the most frequent adverse effects in men. Thus, it would seem to be prudent to include in the label some information on vasculitis in animals.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: **Concurrence -** _____

Non-Concurrence - _____
(see memo attached)

C. Cc: NDA 21-368
HFD-580/D. Spell-LeSane, M. Hirsch, A. Batra, G. Benson, M.-J. Ng, D. Hoberman, S. Roy, V. Jarugula, A. Parekh, M. Rhee, R. Agarwal, Y. Shin, A. Jordan
HFD-510/J. El-Hage, K. Davis-Bruno

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY (see Reviews #1/3/4 for IND 54,553

Primary pharmacodynamics:

Mechanism of action: IC351 inhibits PDE5, a major cGMP-hydrolyzing enzyme in human cavernosal smooth muscle, and enhances relaxant effects of NO by stimulating cGMP levels. This leads to relaxation of penile resistance arteries and cavernosal trabecular smooth muscle (#00-0010-11).

Drug activity related to proposed indication: IC351 enhances the erectile response by smooth muscle relaxation and inflow of blood into the penile tissues.

Secondary pharmacodynamics: IC351 had no direct effect on human platelet aggregation but markedly potentiated the anti-aggregatory effect of soluble guanylyl cyclase activator sodium nitroprusside (SNP) in a dose-dependent manner (#97-002-14).

Pharmacology summary: IC351 is a potent inhibitor of PDE5 among 10 PDEs (700- to 49,000-fold) *in vitro* (#00-0006-11). IC351 displays >10,000-fold selectivity for PDE5 against PDE3A found in cardiac myocytes and 700-fold selectivity against PDE6 in photoreceptor. PDE5 was not present in human cardiac myocytes (#00-0009-11). The intermediate derivatives of IC351 (catechol and methylcatechol) and the methylcatechol glucuronide metabolite were 45- and 230-fold less potent than IC351 with some selectivity for PDE5. The major circulating metabolite in human plasma and urine, methylcatechol glucuronide, displays 13,000-fold less potency than IC351 as a PDE5 inhibitor, but does not show selectivity. Sponsor stated that methylcatechol glucuronide would not have clinically significant effects on any of the human PDEs at concentrations achieved in human plasma following efficacious oral doses (#01-0007-11).

Pharmacology conclusions: IC351 strongly potentiated the inhibitory effects of SNP on human platelet aggregation with complete inhibition at 0.25 μM, and increased cGMP levels in the presence of SNP in human cavernosal smooth muscle, suggesting that the PDE5 inhibition by IC351 may lead to large increases in cGMP levels once activated.

II. SAFETY PHARMACOLOGY (see Reviews #1 for IND 54,553

Safety pharmacology summary: IC351 produced emesis/tachycardia at ≥30 mg/kg (p.o.) in conscious dogs (1/2 at 30 mg/kg and 2/2 at 100 mg/kg) and ptosis/depression of the pinnal reflex at 200 mg/kg in rats. Decrease in mean arterial blood pressure was observed at ≥1 mg/kg (p.o.) in hypertensive/normotensive rats and ≥20 mg/kg (p.o.) in conscious dogs. IC351 at cumulative i.v. doses of 0.1 to 3 mg/kg produced dose-dependent decreases in blood pressure in anesthetized dogs secondary to decreased vascular resistance. Administration of IC351 to conscious guinea pigs at an oral dose of 400 mg/kg produced significant reduction in heart rate and progressive bradycardia, concurrent with deteriorating clinical signs and weight loss resulting in death (#S21422). IC351 potentiated ANF-induced diuresis and natriuresis in rats at 0.1 mg/kg (i.v.). IC351 had affinity for the D_2 receptor at 1 μM.

Safety pharmacology conclusions: The effective antihypertensive oral doses of IC351 were 1 mg/kg in the rat and 20 mg/kg in conscious dogs.

III. PHARMACOKINETICS (see Reviews #1/3/4 for IND 54,553

PK parameters: PK of IC351 are linear with respect to time and dose in healthy subjects or in patients with ED. Exposure (AUC) increased proportionally over a dose range of 2.5- to 20 mg. C_{max} was

achieved at a median time of 2 hrs after dosing. Steady-state plasma concentrations were attained by Day 5 with doses of 10- or 20 mg/day with a $t_{1/2}$ of 17.5 hours.

Absorption: Absorption of IC351 is generally rapid in rats and dogs with T_{max} of 1 to 2 hours and 6 hours with oral administration of 10 mg/kg [^{14}C]-IC351, respectively. Repeated oral dosing caused a variable and prolonged T_{max}, suggesting the possibility of absorption in the lower intestinal tract. Oral bioavailability at 10 mg/kg was 34-53% in rats and 10-18% in dogs. Plasma half-life after oral administration in rats and dogs could not be calculated due to the limited number of data points and a prolonged absorptive phase.

Distribution: An oral dose of [^{14}C]IC351 (10 mg/kg) to rats revealed the highest concentrations of radioactivity in the stomach, GI tract, thyroid and lung. By 168 hrs after dosing, radioactive drug-related material was not detected in any tissues except for the liver. Exposure to pregnant rats on gestation Day 18 caused the highest concentrations of radioactivity in maternal adrenal gland, preputial gland and liver at 8 hours post-dose. Parent and/or metabolites of IC351 were detected in the maternal placenta, and fetal adrenal gland, blood, brain, eye, kidney, liver & myocardium with substantially low exposure at 8 hours post-dose, indicating the placental transfer.

Metabolism: IC351 is predominantly metabolized by CYP3A4 in human liver. Unchanged IC351 accounted for 26% in human plasma, indicating an extensive metabolism. In human feces, the majority of the radioactivity was associated with metabolites after 24 hours. In human, dog and mouse liver slices, the major metabolite was the methylcatechol glucuronide. Catechol glucuronide was the most abundant metabolite in rat liver.

Excretion: Major route of elimination of a radiolabled dose (100 mg) was the feces with 61% and urine with 36% in human by 13 days. Elimination of radioactivity in feces at a single dose of 10 mg/kg was 98% in rats, 84% in male dogs and 63% in female dogs. Only ≤0.1% of administered IC351 is excreted in maternal milk over a 3 to 24 hour period, suggesting that maternal milk is not a major route of elimination for IC351 and metabolites.

Protein binding: *In vitro* plasma protein binding to mouse, rat, dog and human was determined to be 85%, 92%, 87% and 94%, respectively.

PK/TK summary: Plasma concentrations generally increased sub-proportionally to the increased dose in mice, rats and dogs. Exposure was higher after 1 to 3 months in rats and dogs, suggesting accumulation in the plasma. The plasma exposure in pregnant rats following daily oral dosing from gestation Days 6 through 12 was also less than proportional to dose over the range of 60 to 1000 mg/kg with T_{max} of 4 to 12 hours. The only gender difference was a higher exposure in female rats, and is not marked in humans.

Summary of $AUC_{(0\text{-}24h)}$ in Subchronic- and Chronic Studies for Mice, Rats and Dogs

Species	Study #	Duration		Dose, mg/kg	$AUC_{(0\text{-}24h)}$, ng·h/mL	
					M	F
Mouse (CD-1)	CTBR88440	3 Months	Day 90	60	18231	13390
				200	19701	26275
				400	26860	27152
	M04398	3 Months	Day 90	60	7886[a]	13492[a]
				200	17822[a]	22699[a]
				400	18559[a]	19827[a]
				800	20004[a]	22421[b]
	CTBR88780	6 Months (Carcinogenicity)	Day 180	10	7125[c]	7023[c]
				60	14999[d]	12062[d]

				400	31223[d]	20962[d]
Rat (Wistar)	R20861	1 Month	Day 27	10	13280[e]	25010[e]
				60	54120[e]	74320[e]
				400	83910[e]	159600[e]
	R18498 (Fisher)	3 Months	Day 90	60	29427	46354
				100	21221	51802
				400	49951	126171
				800	41649	77182
	R21236	6 Months	Day 168/169	10	14900[e]	28200[e]
				60	29100[e]	82900[e]
				400	72200[e]	190000[e]
	CTBR88779	6 Months (carcinogenicity)	Day 180	10	16070	35899
				60	38604	91106
				180	78863	152863
Dog (beagle)	D20863	1 Month	Day 28	10	5060-11100	2390-4160
				45	6750-7420	15300-33200
				200	209000-230000	131000-138000
	88270	3 Months	Day 91	10	2565-14553	-
				60	11907-21669	-
				200	35629-74920	-
	CTBR88632	6 Months	Day 176	10	NC-6882	NC-6202
				60	13370-25863	11424-44885
				200	13007-62645	16983-55001
				400	31384-91270	41786-129341
	D21235	6 Months	Day 182/183	10	4350-43200	4960-26900
				60	13100-119000	36600-98600
				400	68300-179000	44300-261000
	D01899	1 Year	Day 364	25	8576-43136	8792-68012
				100	12122-62109	39276-158317
				400	16900-77350	28936-109961
Human	LVDK		Day 5	20 mg	7692	-

[a]$AUC_{(0\text{-}12h)}$, [b]$AUC_{(0\text{-}16h)}$, [c]$AUC_{(0.5\text{-}\tau)}$, [d]$AUC_{(0\text{-}\tau)}$, [e]$AUC_{(1\text{-}24h)}$

PK/TK conclusions: Metabolism was the primary mechanism for clearance of IC351 from the systemic circulation with similar routes of biotransformation in mice, rats, dogs and humans. The major route of excretion was via the feces in both rats and dogs, indicating incomplete oral absorption and biliary excretion of metabolites. Slight- to moderate increases in hepatic enzyme activity and/or CYP450 content were observed in mice, rats and dogs after oral doses of ≥400 mg/kg, indicating IC351 as a inducer of CYP450 isoenzymes.

IV. TOXICOLOGY (see Reviews #1/2/3 for IND 54,553

Study title: 1-Year Oral Toxicity Study in Beagle Dogs

Key study findings: Testicular degeneration/atrophy was observed with deceased sperm in the epididymides at all treatment groups with increased severity following 12 months. Neutropenia, thrombocytopenia and/or anemia were observed in the mid- and high dose females (1/5 each group).

Study no: D01899 **Volume #, and page #:** vol. 31
Conducting laboratory: Eli Lilly & Company, Greenfield, IN 46140
Date of study initiation: June 19, 1999 **GLP compliance:** Yes
QA report: yes (x) no ( )
Drug/lot #: IC351 (LY450190)/991020 **% purity:** 100.1%
Formulation/vehicle: Gelatin capsule/1% (w/v) carboxymethylcellulose sodium & 0.5% sodium lauryl sulfate in purified water

Dosing:

Species/strain: Beagle dog
#/sex/group or time point (main study): 5/sex/group
Satellite groups used for toxicokinetics or recovery: no recovery studied
Weight: 9.0 to 12.9 kg for males & 7.3 to 9.9 kg for females **Age:** 14 to 15 months

Doses in administered units: 0, 25, 100 & 400 mg/kg/day
Route, form, volume, and infusion rate: Oral suspension of 0, 12.5, 50.0 & 200.0 mg/mL in capsules

Observations and times:

Observations	Times
Mortality/Moribundity Clinical Signs	More than once daily
Body Weights/Food Consumption	Twice daily pre-study phase/Once weekly post-dose phase
Ophthalmoscopy	Pretreatment & terminal examination
Pathology/Urinalysis/Organs Weighs	Scheduled necropsy on Days 365 & 366
Electrocardiography	Day -7/pre-dose & 2 hrs post-dose on Days 180 & 362
Clinical Pathology	Twice daily pre-study phase/Months 1, 3, 6, 9 & 12 for fasted Months 5, 8, 10, 11 & 12 for non-fasted
Toxicokinetics	0, 1, 2, 4, 8, 12, 16 & 24 hrs on Days 0, 33, 177 & 364

Results: Treatment was suspended for one 100- & one 400 mg/kg female dogs between Days 140 and 166 & from Days 196 through the end of the study due to marked neutropenia (1,610/µL for 100 mg/kg & 310/µL for 400 mg/kg compared to reference 3,300 to 11,600/µL) with moderate thrombocytopenia (90,000/µL for 100 mg/kg & 226,000/µL for 400 mg/kg compared to reference levels of 191,000 to 442,000/µL), which was initially identified on Day 91. Severe neutropenia (170/µL), moderately decreased platelets (111,000/µL), hyperglobulinemia, minimal anemia, minimal eosinopenia and ↑ALP in the absence of clinical signs were present in the 100 mg/kg dog on Day 140. The other blood dyscrasias included minimal monocytopenia, lymphopenia & eosinopenia in both dogs, which the sponsor considered to be due to stress. Clinical signs of fever (105.4°F), anorexia & lethargy followed by minimal neutropenia (2,930/µL), anemia, severe monocytosis & slight hyperglobulinemia compatible with inflammation occurred in the 400 mg/kg dog on Day 128. Abdominal radiographs taken on Days 128 & 288 excluded an occult inflammatory focus or splenic enlargement. Antibiotic therapy was initiated on Day 128 due to signs of inflammation (↑basophilia/Dōhle bodies). After removal from antibiotics on Day 138, clinical signs returned to normal with severe neutropenia (200/µL) & neutrophil alterations. The dog became febrile & developed neck pain/inappetence on Day 141 despite the reinstatement of antibiotic on Day 139. Additional aspirin & antibiotics were administered on Days 146 through 154. Following drug removal (& antibiotic & supportive therapy in the 400 mg/kg) on Days 140 (400 mg/kg) & 142 (100 mg/kg), dosing resumed on Days 161 (400 mg/kg) & 166 (100 mg/kg). Marked neutropenia (1,130/µL for 100 mg/kg & 1,210/µL for 400 mg/kg) and/or minimal thrombocytopenia developed within 8 days in the 400 mg/kg or within 10 days in the 100 mg/kg dog. These dogs were clinically asymptomatic until Days 195 when the 400 mg/kg dog developed clinical signs of stiffness/neck pain/anorexia, neutrophilia (10,250/µL), monocytosis & hyperglobulinemia. Discontinuation of IC351 on Day 196 returned the neutrophil (Day 204 for 100 mg/kg & Dat 196 for 400 mg/kg) and/or platelet counts (Day 208 for 100 mg/kg) within the reference interval & without clinical signs. Bone marrow aspirates and core biopsies from humerus taken from these 2 dogs on Day 196 demonstrated no abnormalities but increased numbers of immature neutrophilic precursors and myeloid/megakaryocytic hyperplasia, which were less pronounced on Days 231 (100 mg/kg) or 285 (400 mg/kg) and absent on Day 366. Sponsor considered the findings to be idiosyncratic and not a result of a direct compound-related effect on early neutrophil precursors or on bone marrow. The high-dose female had a single focus of perivasculitis in the circumflex branch of the left coronary artery. The 100 mg/kg dog showed a pituitary neuroblastoma, which was considered to be spontaneous.

Drug-related degeneration & atrophy of the seminiferous epithelium occurred in dogs at ≥25 mg/kg with decreases in testicular weight & gross findings of small/soft testes. Degeneration of the seminiferous

epithelium was characterized by disassociation or vacuolation of seminiferous epithelium, mutinucleated cells, exfoliated germ cells, pyknotic spermatids, megalospermatids, attenuation or loss of cell layers, and/or increase in Leydig cells. Low numbers of tubules also had markedly enlarged cells of spermatogonia with abundant eosinophilic cytoplasm & round nuclei with rarefaction of the chromatin. Scattered tubules had individual cell necrosis of the mitotic spermatocytes and/or elongating spermatids. Atrophy of the seminiferous epithelium was characterized by seminiferous tubules lined by only Sertoli cells and/or spermatogonia. Degeneration and atrophy were more pronounced in the periphery of the affected testes. These testes were decreased overall in diameter with obliteration of the lumen or attenuation of the epithelium with increased intraluminal space compared to controls. Aspermia were also noted, which was considered secondary to decreased testicular sperm production.

Dose, mg/kg, n=5	0	25	100	400
Testes weight (g), absolute	12.68	11.38	10.00	8.22*
Epididymides weight (g), absolute	3.00	3.10	2.60	2.68
Testes, small		1	2	4
Testicular seminiferous epithelium				
Bilateral degeneration	0	2 minimal 2 slight 1 severe	3 slight 2 moderate	1 slight 1 moderate 3 severe
Bilateral atrophy	0	1 marked	1 minimal 2 slight	1 marked 2 severe
Epididymis				
Bilateral aspermia	0	1	0	2
Bilateral multifocal epithelial vacuolation	1 minimal	1 minimal	2 minimal	2 slight

*Significantly different from control at $p \leq 0.05$

Table below summarizes the results for the rest of the animals except the 2 female dogs with marked neutropenia. No drug-related arteritis lesions occurred in the present study, due perhaps to lower blood drug levels or different dog colony. No drug-related mortality was observed. Abnormal feces were more frequently observed in all treated groups. There was dose-dependent decrease in mean body weight in females, which was observed by 6 weeks after study initiation, & maintained without additional changes. WBC parameters of reticulocytes, neutrophils and monocytes were decreased from mid dose. IC351 produced slight dose-dependent increases in CYP450 content in males with increased liver weight. Increased adrenal weight was observed with enlarged/mutifocal agonal hemorrhage and multifocal accessory structure in a high dose male dog. Slightly increased incidence of hepatic leukocytosis/centrilobular pigmentation was found in the high dose group. Sponsor considered these & all other findings spurious due to the small magnitude of the change, individual variablity & overlap with concurrent controls. Plasma exposure increased on repeated dosing with marked variability within the same treatment group. Half-life was not calculated due to the limited number of time points. TK data were taken directly from the submission.

Dose, mg/kg	0		25		100		400	
	5M	5F	5M	5F	5M	4F	5M	4F
Mortality	0	0	0	0	0	0	0	0
Clinical signs, n=5								
Feces, pale	0	0	4	3	5	5	5	5
Lameness	0	0	0	0	0	0	2	0
Skin, laceration/abrasion/red	0	0	0	0	0	0	1	0
Skin, swollen	0	0	1	0	0	0	1	0
Digit, swollen	0	0	1	0	0	0	1	0
Decreased activity	0	0	0	0	0	0	0	1
Vaginal discharge, brown	na	0	na	0	na	0	na	1
Mammary gland, swollen	0	0	0	1	0	1	0	1

Body weights, kg, Day 364	12.36	11.30	12.58	**10.48**	12.28	**9.40**	12.54	**9.55***
Food consumption	UR	UR	UR	UR	UR	UR	UR	UR
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR
Hematology, Day 363								
Reticulocytes, $10^3/\mu L$	68.4	80.6	83.4	105.6	73.0	**56.5**	**43.0**	**42.8****
Neutrophils, $10^3/\mu L$	7.036	7.694	6.290	5.174	6.804	5.598	**5.812**	**6.305**
Monocytes, $10^3/\mu L$	0.578	0.668	0.482	**0.340*****	0.568	**0.450***	**0.466**	**0.610****
Clinical chemistry, Day 363								
Cholesterol, mg/dL	150.84	206.7	163.86	225.84	156.02	190.90	**183.70**	202.05
Triglyceride, mg/dL	27.66	53.60	34.24	37.12	29.38	31.88	**43.82**	35.95
Inorganic phosphorus, mg/dL	4.020	3.820	3.480	3.700	3.540	3.075**	3.380	3.525*
Electrocardiography	UR	UR	UR	UR	UR	UR	UR	UR
Urinalysis	UR	UR	UR	UR	UR	UR	UR	UR
Hepatic Microsomal Enzyme,								
Total CYP450, nmol/mg protein	0.389	0.315	**0.411**	0.433	**0.459**	0.426	**0.508***	**0.418**
Organ weights, absolute, g								
Kidneys	53.26	43.90	48.74	37.18*	49.26	**33.23****	53.92	**36.10***
Liver	248.4	298.0	318.2	238.8	**276.4**	228.8	**318.8**	250.5
Adrenals	1.346	1.240	1.427	1.387	1.441	1.380	1.615	1.260
Thyroids	0.678	0.616	0.818	0.643	0.761	0.597	0.861	0.759
Gross pathology,								
Alopecia	0	0	0	0	0	0	0	1
Stomach, lesion	0	0	0	0	0	0	0	1
Liver, lesion	0	0	0	2	1	0	1	0
Spleen, lesion	0	0	0	1	0	1	1	0
Lymph node, enlarged	0	0	0	0	0	0	0	1
Skin, lesion	0	0	0	0	0	0	0	1
Adrenal, enlarged	0	0	0	1	0	1	1	0
Thyroid, enlarged	0	0	0	0	1	0	1	1
Pituitary, enlarged	0	0	0	0	0	0	1	0
small	0	0	0	0	0	0	1	0
Histopathology,								
Liver, diffuse sinusoidal leukocytosis	2	0	3	0	1	0	3	2
subacute multifocal perivasculitis	3	3	1	2	3	3	5	2
centrilobular hepatocellular pigmentation	1	0	1	1	0	1	3	1
Gallbladder, multifocal lymphocytic infiltration	0	0	1	0	1	3	0	1
Lung, chronic focal proliferative bronchiolitis	0	0	0	1	0	0	1	0
chronic multifocal proliferative bronchiolitis	2	2	1	0	2	1	3	1
Spleen, hemosiderosis	0	0	1	0	0	0	1	0
Salivary gland, multifocal lymphocytic infiltration	0	0	1	0	0	0	1	0
Tongue, multifocal lymphocytic infiltration	0	0	0	0	0	0	1	0
Prostate, multifocal atrophy	1	na	4	na	2	na	2	na
multifocal acinar dilation	2	na	4	na	5	na	3	na
multifocal fibrosis	0	na	1	na	1	na	1	na
chronic focal inflammation	0	na	1	na	1	na	1	na
Skeletal muscle, multifocal degeneration	0	0	0	0	0	0	1	0
Adrenal, multifocal cortical accessory structure	0	1	0	0	1	0	1	0
Thyroid, multifocal mineralization	0	0	0	0	0	0	1	0
Parathyroid, focal ductal cyst	0	0	0	0	0	0	0	1
Pituitary congestion	0	0	0	0	0	0	1	0
Cerebrum, acute multifocal agonal hemorrhage	0	0	0	0	0	0	1	0
Brain stem, acute multifocal hemorrhage	0	0	0	0	0	0	1	0

Significantly different from control at $p \le 0.05^*$, $p \le 0.01^{**}$ or $p \le 0.001^{***}$
UR- unremarkable

Compound: IC351 (LY450190)
Study: D01899

Table 1: Summary of Plasma Exposure Parameters in Beagle Dogs after Oral Administration of 25, 100, or 400 mg IC351/kg/day for Up to 1 Year

Parameter	Administered Dose (mg/kg/day)					
	25		100		400	
Sex	Male [a]	Female [a]	Male [a]	Female [b]	Male [a]	Female [b]
Day 0						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	655 ± 499	578 ± 143	708 ± 264	1196 ± 1216	2662 ± 2251	4058 ± 2729
T_{max} (hr)	2 to 16	2 to 4	2 to 12	2 to 24	2 to 24	4 to 16
Day 33						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	737 ± 462	1159 ± 349	1201 ± 565	4255 ± 2233	3159 ± 1862	4168 ± 2168
T_{max} (hr)	2 to 12	2 to 8	2 to 8	0 to 24	2 to 8	2 to 4
Day 177[c]						
Range of $AUC_{1\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	1123 ± 584	1987 ± 1026	1758 ± 928	3690 ± 906	3164 ± 1599	5914 ± 2354
T_{max} (hr)	2 to 16	2 to 12	2 to 8	1 to 16	4 to 12	2 to 12
Day 364						
Range of $AUC_{0\text{-}24\ hr}$ (ng•hr/mL)						
Mean (± SD) C_{max} (ng/mL)	1440 ± 706	2301 ± 1356	2706 ± 1748	4763 ± 2660	3207 ± 1820	5413 ± 2512
T_{max} (hr)	2 to 8	2 to 4	4 to 12	8 to 24	4 to 8	12 to 16

[a]N = 5 dogs/sex; [b]N= 4 dogs/sex as Dog 283863 (100 mg/kg) and Dog 284504 (400 mg/kg) were excluded from calculation of summary statistics as a result of cessation of dosing on multiple periods, for extended lengths of time; ; [c]Time zero plasma sample not collected – AUC calculated from 1 to 24 hours.

Abbreviations: AUC = Area under the plasma concentration-time curve from 0 to 24 hours; C_{max} = maximal observed plasma concentration; SD = standard deviation; T_{max} = range of time to reach C_{max}.

Human $AUC_{0\text{-}24}$ at steady state= 7,700 ng•hr/mL with 20 mg/day (LVDK)

IND No.

Summary: No NOAEL was identified due to testicular findings. Present study was conducted using a CMC/[redacted] instead of IC351: [redacted] used in the previous studies based on a pilot study (Study #D02799; data not provided) as it yielded the most consistent plasma levels and is comparable to the market image according to the sponsor. Plasma exposure to IC351 increased sub-proportionally to the dose with marked variability within the same treatment group possibly due to absorption in the lower intestine. No drug-related mortality was observed. Mean body weight for females in the 100- and 400 mg/kg groups was decreased by 6 weeks after dosing which persisted throughout the study. Increase in weight & histopathological findings in the liver correlated with an increase in total hepatic CYP450. Incidence of small/soft testes and testicular degeneration/atrophy of seminiferous epithelium at all dose groups correlated with concomitant decrease in testis weight and aspermia in epididymides. The incidence tended to be more severe compared to 6-month studies, suggesting that severity increases with chronic dosing. Although the reversibility of the finding is unknown for the 1-year study, extensive cell loss in the germinal epithelium was observed in dogs with the most severe testicular alterations, such that reversibility is unlikely. Sponsor indicated that morphologic alterations such as necrosis of spermatogonia, and Leydig cell hyperplasia and prostatic atrophy, which are suggestive of direct cytotoxicity and disruption of the pituitary-gonadal hormonal axis, respectively, were not observed.

Marked neutropenia & thrombocytopenia were observed in one female dog of the mid- and high dose groups, which was reversible within 2 weeks after removal from the drug. The 400 mg/kg female exhibited anemia, neutropenia, thrombocytopenia & perivasculitis in the circumflex branch of the left coronary artery. The effects in the 400 mg/kg female were accompanied by clinical signs of fever, anorexia & lethargy. Bone marrow samples and sera taken from these 2 dogs on Day 196 demonstrated no detectable anti-RBC antibodies with a modified indirect Coomb's test, but did show an increase in neutrophilic precursors and myeloid/megakaryocytic hyperplasia. Sponsor considered that these findings were idiosyncratic, and not a result of a direct effect on bone marrow hematopoietic precursors. The sponsor also ruled out some potential mechanisms of drug-induced hematologic disorders since (1) there was a relatively short recovery time and localization of effects to the mature neutrophil populations, indicating no direct effects on immature neutrophilic precursors and/or bone marrow stem cells; (2) there were no clinical signs, inflammatory leukograms & neutrophil cytoplasmic changes in the 100 mg/kg dog, suggesting neutrophil consumption with inflammation or sequestration was not responsible for the neutropenia; (3) inflammatory changes (neutrophilia/monocytosis/neutrophil cytoplasmic changes and/or hyperglobulinemia) in the 400 mg/kg dog were limited to periods when the dog exhibited clinical signs; (4) antibiotic & supportive therapy alone failed to resolve neutropenia; and (5) there was no evidence of splenic enlargement, commonly associated with peripheral consumption of blood cells.

TOXICOLOGY SUMMARY AND CONCLUSIONS:

PDE inhibitors are associated with disseminated arteritis and testicular degeneration in dogs and in other animal species. IC351 also caused seminiferous epithelial atrophy of the testis in a dose-dependent manner, which correlated with a decrease in testicular weight and oligo/aspermia in the dog studies. These findings were observed in the 3-month toxicity study and the carcinogenicity study in mice and in the 3-, 6- and 12-month toxicity studies in dogs with low multiple of exposure compared to the human exposure at a dose of 20 mg. The lesions are likely to be non-reversible in dogs, and the severity increased with chronic dosing. There were no clinically significant effects on semen parameters up to 6 months with a dose of 20 mg in 217 men (#H6D-MC-LVCZ).

In dogs, arteritis was observed in multiple tissues including thymus, lung, and spinal cord at high doses with increased incidence from the 1-month study. Coronary arteritis was observed at ≥45 mg/kg in the 1-month study with no hemodynamic effects (vasodilation/tachycardia), suggesting a drug-related effect. In the 3-month study, myocardial degeneration, fibrosis and epicarditis was observed at 200 mg/kg

in a single male dog (1/4). The 6-month study showed slight increased incidence and severity of disseminated periarteritis at 400 mg/kg in multiple tissues including coronary arteries. The periarteritis was associated with medial/epicardial/subendothelial inflammatory cell infiltration, neutrophilic adventitial inflammation and medial fibrinoid necrosis. The one-year dog study did not duplicate the vasculitis findings observed in the previous dog studies. The reason may be due in part to different dog colony or lower drug exposures. One high dose female dog exhibited a single focal perivasculitis in the circumflex branch of the left coronary artery and marked neutropenia/thrombocytopenia, anemia and hyperglobulinemia with fever, lethargy and anorexia. Another female in the mid-dose group developed moderate neutropenia/thrombocytopenia, and treatment for both dogs was suspended during the study. No other lesions for drug-induced vasculitis of atrial epicardial hemorrhage or myocardial necrosis were observed. Sponsor considered that the findings for the 2 dogs were drug-related idiosyncratic hematologic disorders. The NOAEL for the vasculitis findings produced approximately 1- to 3-fold exposure multiples for 1-month study at 10 mg/kg and 3- to 33-fold exposure multiples for 6-month study at 60 mg/kg of unbound parent drug (due to individual variability) to humans taking 20 mg. Periarteritis and hemorrhage/necrosis of lymphoid were also observed in mice and rats with moderate safety margins (<10 fold) at the NOAEL.

Daily oral administration of IC351 to rats for 6 months was generally well tolerated up to 400 mg/kg without drug-related effects on mortality or body weight. Brown pigment deposition in the cytoplasm of periportal hepatocytes associated with focal accumulation of Kupffer cells was observed with increased liver weight in female rats given 60 and 400 mg/kg. The hepatocellular pigmentation was also observed in male dogs of the 3- and 12-month studies at 200 and 400 mg/kg, respectively. Phlebitis of mesenteric beds at all treated groups and hepatic arteritis in high dose rats were noted. Other histopathological findings included renal tubular epithelial regeneration/hyperplasia, splenic extramedullary hematopoiesis or hemorrhage in the lymph node/thymus with increased frequency in the high dose group.

IC351 administered at doses up to 800 mg/kg/day for 3 months in mice produced no treatment-related deaths or body weight changes. Periarteritis in the testicular/mesenteric arteries was observed with lymphocytic atrophy/necrosis in the spleen/thymus of the high dose group. These lesions were correlated with epididymal bilateral epithelial vacuolation or prostatic chronic focal inflammation. Other microscopic findings included splenic hematopoiesis in all treated groups.

Labeling Recommendations under Animal Toxicology:

Histopathology Inventory for NDA #21-368

Study	1-mo	1-mo	3-mo	3-mo	3-mo	6-mo	6-mo	1-yr	2-yr	2-yr
Species	Rat	Dog	Mouse	Rat	Dog	Rat	Dog	Dog	Mouse	Rat
Adrenals	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Aorta	X	X	X	X	X	X	X	X	X	X
Bone Marrow smear	X	X	X	X	X	X	X	X	X	X
Bone (femur)	X	X				X	X	X		
Brain	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Cecum	X	X	X	X	X	X	X	X	X	X
Cervix			X	X				X		
Colon	X	X	X	X	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X	X*	X*	X	X
Esophagus	X	X	X	X	X	X	X	X	X	X
Eye	X	X	X	X	X	X	X	X	X	X
Fallopian tube										
Gall bladder		X	X		X		X	X	X	
Gross lesions	X	X	X	X	X	X	X	X	X	X
Harderian gland	X		X	X		X			X	X
Heart	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Ileum	X	X	X	X	X	X	X	X	X	X
Injection site										
Jejunum	X	X	X	X	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Lachrymal gland		X	X		X		X	X	X	X
Larynx	X	X	X	X	X	X	X			
Liver	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Lungs	X*	X*	X*	X	X	X*	X	X	X	X
Lymph nodes, cervical	X	X		X	X	X	X	X		
Lymph nodes mandibular			X				X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X	X	X	X	X
Nasal cavity										
Optic nerves	X	X	X		X	X	X		X	X
Ovaries	X*	X*	X*	X*		X*	X*	X*	X	X
Pancreas	X	X	X	X	X	X	X	X	X	X
Parathyroid	X	X*	X*	X*	X*	X	X*	X*	X	X
Peripheral nerve	X	X	X	X		X				
Pituitary	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Prostate	X*	X*	X*	X*	X*	X*	X*	X*	X	X
Rectum	X	X	X	X	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X	X	X	X	X
Sciatic nerve			X		X			X	X	X
Seminal vesicles	X		X	X		X			X	X
Skeletal muscle	X	X	X	X	X	X	X	X	X	X
Skin	X	X	X	X	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X*	X*	X	X	X
Sternum	X		X	X	X	X	X			
Stomach	X	X	X	X	X	X	X	X	X	X
Testes	X*	X*	X*	X*	X*	X	X*	X*	X	X
Thymus	X*	X*	X*	X*	X*	X*	X*	X	X	X
Thyroid	X	X*	X*	X*	X*	X	X*	X*	X	X
Tongue	X	X	X	X	X	X	X	X	X	X
Trachea	X	X	X	X	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X	X	X	X	X
Uterus	X	X	X*	X	X	X	X*	X	X	X
Vagina	X	X	X	X	X	X	X	X	X	X
Zymbal gland	X					X				
Hepatic artery			X	X		X	X	X		
Mesenteric artery & vein			X	X		X	X	X		
Testicular artery			X	X		X	X	X		
Coronary artery		X					X	X		

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY (see Reviews #1 for IND 54,553

IC351 was not genotoxic or mutagenic in *in vitro* bacterial Ames test, mammalian cell mutation assay or cytogenetic study in human lymphocytes, and not clastogenic in *in vivo* rat micronucleus assay.

Labeling recommendations:

VI. CARCINOGENICITY:

Study title: 2-Year Oral Gavage Carcinogenicity Study of IC351 in Albino Mice

Key study findings: Degeneration/atrophy of testicular tubular epithelium associated with oligo/aspermia in the epididymis was slightly increased at ≥60 mg/kg. Hepatocellular adenomas and alveolar/bronchiolar adenomas/carcinomas increased in the high dose males and females, respectively, but was not statistically significant. The AUC of the high dose (males and females) for the unbound parent drug was approximately 10 times the human AUC at the proposed clinical dose of 20 mg.

Study number: 88455 — 88780 for — **Volume #, and page #:** vol. 33
Conducting laboratory and location:
Date of study initiation: December 19, 1997
GLP compliance: yes **QA report:** yes (x) no ( )
Drug: IC351 (LY450190,) **Lot # (% purity):** F96/048A (47.0%), 43582 (47.1%)
CAC concurrence: Dose selection was not reviewed by the Executive CAC but the committee concurred on the ongoing studies on 6/16/99 based on AUC ratios with a 10 mg human dose (see Appendix II for report).

Study Type: 2-year rodent bioassay
Species/strain: Crl:CD^{R}-1(ICR) mice (*Mus musculus*) **Number/sex/group:** 50/sex/group
Age at start of study: 6 weeks (24.3-32.8 g for males and 19.2 to 25.6 g for females)
Animal housing: Individual
Formulation/vehicle: 0.5% hydroxypropyl methylcellulose (HPMC) containing 1% Tween 80
Drug purity/stability/homogeneity: Accessed
Methods:
Doses: 0, 10, 60 & 400 mg/kg **Basis of dose selection:** AUC ratios
Route of administration: Oral gavage **Frequency of drug administration:** Daily
Dual controls employed: Dual identical controls **Interim sacrifices:**
Satellite PK or special study group(s): 3/sex/timepoint for PK
Deviations from original study protocol: N/A
Statistical methods: Proc Multtest implemented with the Peto's survival-adjusted one-sided trend test
Observations and times:

Observations	Times
Mortality/Clinical Signs	Twice daily/Detailed physical exam weekly
Body Weights/Food Intake	Weekly
Food Consumption	Weekly/Monthly after 13 weeks
Ophthalmology	Weeks -1, 52 & 104
Pathology/Clinical Chemistry	Week 104
Toxicokinetics	Days 21, 84 & 180 at 0, 0.5, 1, 2, 4, 8, 16 & 24 hrs post-dose

RESULTS: Mortality (~50%) was similar in the control and treated groups at the end of the study, although the males in the vehicle group 1 had slightly higher mortality rate from 56- to 100 weeks (see Appendix III for graphical presentation). Major cause of death was urinary tract disorders (inflammation/retention) accounting for 35% in males and lymphoreticular neoplasia in 34% of preterminal euthanized females. There were no treatment-related differences in group mean body weight or food consumption in all groups, and occasional statistically significant differences were not considered to be of biological significance (see Appendix IV for graphical presentation). Clinical signs

included fur staining, scabbing/reddening of the skin and ocular opacities in addition to the decreased activity, dehydration, prominent backbone and masses observed in sacrificed mice, and were considered unrelated to treatment. Plasma exposure was less than proportional to the dose. There were no consistent gender-related differences in the exposure. The half-life could not be calculated due to the limited number of timepoints in the log-linear phase of the plasma concentration versus time curve.

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	25	23	24	25	23	21	23	25	20	26
Clinical Signs,										
Fur thin cover, limbs/paws	4	8	6	8	5	9	10	8	9	6
Fur ungroomed	17	11	3	14	9	11	17	19	15	18
Abnormal breathing, labored/shallow	9	9	9	10	7	6	8	6	15	16
Hunched posture	5	5	5	5	2	1	3	3	9	4
Abnormal feces, decreased/absent	9	5	7	4	4	7	5	6	6	9
Body Weights	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Food Consumption	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Hematology	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Gross pathology	See below		See below		See below		See below		See below	
Toxicokinetics, 3/sex/timepoint										
AUC_{0-t} (ng•hr/mL) Day 21					6044*	6776*	19773	18131	29250	32790
Day 84#					5361$	5697	14232	22039	34023	32501
Day 180					7125*	7023*	14999	12062	31223	20962
C_{max} (ng/mL) Day 21					1584	2404	3379	3246	4617	5259
Day 84#					1264	1175	2306	2965	4324	4452
Day 180					1164	1158	1943	2197	5372	3653
T_{max} (hr) Day 21					1.0	2.0	2.0	2.0	1.0	4.0
Day 84#					1.0	1.0	4.0	1.0	4.0	4.0
Day 180					2.0	2.0	1.0	2.0	1.0	2.0

#Blood collection schedule was limited on Day 84 & only partial exposure profiles were obtained.
*$AUC_{0.5-t}$ due to no quantifiable concentration at time 0.
$$AUC_{1.0-t}$ due to no quantifiable concentration at time 0.
UR- unremarkable
Human AUC_{0-24} at steady state= 7,700 ng•hr/mL at 20 mg/day

Non-neoplastic findings: Sponsor stated that there were no treatment-related non-neoplastic findings (see Appendix V for incidence of histopathology findings). However, increased episode of penis protrusion in the high dose group was consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, which were the major cause of death and preterminal euthanasia accounting for 35% in males. Gross lesion of soft testes was also associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia at ≥60 mg/kg. Increased frequency was observed for the eye opacity in the mid- and high dose males and corneal mineralization/erosion/ulceration in the high dose females. Dark areas and foci in the cecum were more frequently found at high dose males. Edema of the cecum/rectum was observed with increased incidence in the high dose females, which paralleled the amyloidosis in the digestive tract. Histopathological findings in the hematopoietic system and lymphoid were generally associated with various inflammatory process such as dermatitis, cystitis or pyelonephritis. Extramedullary hemopoiesis observed in the liver, adrenal glands, and lymph nodes also paralleled the myeloid hypercellularity in the bone marrow and hemopoiesis in the spleen. Table below summarizes the microscopic findings with increased incidence.

Incidence of Non-neoplastic Findings in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Bulbopenis, hemorrhage	0	na	5	na	4	na	6	na	9	na
inflammation	0	na	1	na	0	na	2	na	5	na
Cecum, deposits, pigment	5	1	10	4	5	3	6	1	13	6
edema	3	1	1	5	0	6	2	3	0	9
Adrenal, angiectasis	0	0	0	0	0	0	0	0	1	1
Epididymis, oligo/aspermia	20	na	17	na	17	na	23	na	24	na
Eye, erosion/ulceration, cornea	0	4	0	1	3	4	2	6	1	8
Kidney, necrosis, papilla	0	1	1	0	0	0	1	0	0	2
infiltration, mixed cell	1	0	4	0	1	1	2	0	1	3
inflammation, interstitial	9	3	1	5	3	1	4	4	6	5
dilatation, tubular	0	0	0	2	1	0	0	0	0	2
Lymph node, mesenteric, hemopoiesis	7	5	0	9	9	11	7	12	3	5
angiectasis	1	0	0	0	0	1	0	1	1	2
Pituitary, cyst	0	1	0	1	0	3	2	1	1	1
Rectum, edema	1	1	0	3	0	0	0	0	0	6
erosion/ulceration	0	0	0	0	0	0	0	1	1	1
Spinal cord, cervical, hemorrhage	0	0	0	0	0	0	0	1	0	1
Testis, atrophy, seminiferous epithelium	22	na	21	na	21	na	24	na	27	na
Subcutaneous tissue, hemorrhage	0	0	0	0	0	1	0	1	1	1
Testis, atrophy, seminiferous epithelium	22	na	21	na	21	na	24	na	27	na
Uterus, thrombosis	na	4	na	0	na	4	na	4	na	5

na- not available

Amyloidosis occurred in adrenal, heart, kidney, lacrimal gland, liver, mesenteric lymph node, ovary, parathyroid gland, spleen, thyroid, uterus, vagina and GI tract with higher episodes in the mid- to high dose females. Sponsor considered these incidences are a multisystemic disorder frequently seen in mice, within normal biological variation and no toxicological significance (no historical range provided).

Incidence of Amyloidosis in Various Organs in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Adrenal	1	1	1	0	2	0	0	1	0	3
Cecum	0	0	1	0	0	0	0	1	0	3
Colon	0	0	1	0	0	0	0	0	0	2
Duodenum	1	0	1	0	1	0	0	1	0	6
Heart	0	0	0	0	0	0	0	2	0	3
Ileum	3	1	1	0	2	0	1	1	0	10
Jejunum	0	0	1	0	0	1	0	1	0	6
Kidney	1	2	1	1	1	5	1	3	0	6
Lacrimal gland	1	0	0	1	0	0	0	0	0	2
Liver	0	1	2	0	2	2	4	0	1	2
Lymph node, mesenteric	0	0	0	0	0	1	0	0	1	1
Ovary	na	1	na	0	na	0	na	3	na	5
Parathyroid	0	0	2	0	1	0	0	2	0	3
Rectum	0	0	0	0	0	0	0	0	0	1
Spleen	0	1	3	0	2	0	3	2	0	2
Stomach	1	0	1	0	0	0	0	2	0	4
Thyroid	0	1	4	0	1	0	1	2	0	4
Uterus	na	1	na	2	na	0	na	3	na	6
Vagina	na	0	na	0	na	0	na	0	na	1

Combined (all organs)	na	**5**	na	**3**	na	**6**	na	**12**	na	**11**

na- not available

Neoplastic findings: None of the individual tumor incidence had either statistically positive trend (p=0.005 for common tumors or p=0.025 for rare tumors) or difference (p=0.01 for common tumors or p=0.05 for rare tumors) compared to each control group (background rate of ≤ 1% for rare tumors). Hemangiosarcomas were found in various organs with increased combined incidence for all treated groups compared to controls, but were not dose-related. The incidence for the high dose group was not statistically significant compared to each control group. Sponsor stated that the tumors were were within the ranges reported in the literature.

Incidence of Primary Hemangiosarcomas in Albino Mice

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Abdomen	0	1	0	0	0	0	0	1	0	0
Jejunum	0	0	0	0	0	1	0	0	0	0
Liver	0	0	0	1	2	1	0	0	0	3[a]
Lymph node, mesenteric	0	0	0	0	0	0	0	0	1	0
Ovary	na	0	na	0	na	1	na	0	na	0
Spleen	0	0	1	0	1	0	3	1	1	0
Uterus	na	0	na	0	na	2	na	1	na	0
Total	**0**	**1**	**1**	**1**	**3**	**5**	**3**	**3**	**2**	**3**

na- not available

[a]p=0.0734 compared to control 1 with pairwise test

Findings of mass/area raised in the liver at 400 mg/kg correlated with hepatocellular adenomas/ carcinomas more frequently in males, and either individual or combined incidence was not statistically significant compared to each control group.

Non-neoplastic/Neoplastic Findings in the Liver in Albino Mice

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Area raised	3	na	9	na	6	na	8	na	5	na
Mass	14	na	10	na	17	na	13	na	24	na
Cyst, biliary	0	1	0	0	1	2	1	0	1	1
Hepatocellular adenomas	13	3	15	0	18	2	12	2	25	1
Hepatocellular carcinomas	1	0	4	1	4	0	6	0	5	1
Carcinomas with adenomas	0	0	2	0	1	0	3	0	2	0
Hemangiosarcoma[#]	0	0	0	1	2	1	0	0	0	3
Multiple tumor-bearing animals	2	1	4	1	7	0	4	1	6	0
Adenomas+Carcinomas	14	3	17	1	21	2	15	2	28[a]	2

na- not available

[a]p=0.0209 from control 1 with pairwise test

[#]Rare tumors

Increased alveolar/bronchiolar carcinoma or combined adenoma/carcinoma in the lung was observed from high dose females. The incidence was statistically non-significant with the one-sided trend test compared to each control. However, pairwise comparison revealed a statistical difference at p=0.01 for combined adenoma/carcinoma for the mid- and high dose compared to control group 2 (but not control group 1).

Non-neoplastic/Neoplastic Findings in the Lung in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	49F	50M	50F	50M	50F	50M	50F
Hyperplasia, bronchioalveolar	1	2	2	4	1	2	5	1	4	3
Adenoma, alveolar/bronchiolar	7	12	16	3	10	9[a]	10	11[a]	10	12[a]
Carcinoma, alveolar/bronchiolar	6	1	8	3	2	4	6	7	5	6[b]
Adenomas+Carcinomas	13	13	24	6	12	13	16	18[*]	15	17[*]

[a]p=0.0444 (trend test) or p=0.0913 (LD), p=0.0167 (MD) and p=0.0132 (HD) with pairwise test from control 2
[b]p=0.0433 compared to control 1 with pairwise test
[*]Significantly different from control 2 at p=0.0053 (MD) and p=0.0099 (HD) with pairwise test

Leiomyosarcoma in the uterus was observed with 8% incidence (4/50) in the high dose group with frequent cystic endometrial/stromal hyperplasia. The incidence was not statistically significant compared to each control group. Kidney tubular cell adenoma/carcinoma was associated with frequent incidence of pelvis dilatation and nephropathy. Renal tubular hyaline droplets correlated with systemic neoplasm histiocytic sarcoma. Other rare tumors included malignant meningioma in the brain/optic nerve/pituitary, malignant luteoma in the ovary, islet cell adenoma in the pancreas or squamous cell carcinoma in the stomach observed infrequently (1/50) in the high dose females.

Other Neoplastic Findings in Albino Mice

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, benign pheochromocytoma	0	0	0	0	0	0	0	0	0	1
malignant pheochromocytoma	1	0	0	0	1	0	0	0	1	0
Brain, malignant meningioma[#]	0	0	0	0	0	1	0	0	0	1
Hemolymphoreticular tissue,										
histiocytic sarcoma	1	4	0	9	0	10	1	4	0	5
malignant lymphoma	4	6	1	8	6	8	5	10	3	4
Kidney, adenoma, tubular cell	0	0	0	0	1	0	1	0	2	0
carcinoma, tubular cell[#]	1	0	0	0	1	0	0	0	1	0
Optic nerve, malignant meningioma[#], metastasis	0	0	0	0	0	1	0	0	0	1
Ovary, cystadenoma	na	0	na	2	na	2	na	2	na	2
malignant luteoma[#]	na	0	na	0	na	1	na	0	na	1
Pancreas, adenoma, islet cell[#]	0	0	0	0	0	1	0	0	0	1
Pituitary, adenoma	0	2	0	1	0	0	0	3	1	0
malignant meningioma[#], metastasis	0	0	0	0	0	0	0	0	0	1
Spleen, sarcoma[#], metastasis	0	0	0	0	0	1	0	1	0	1
Stomach, carcinoma, squamous cell[#]	0	0	0	0	0	0	0	0	0	1
Testis, adenoma, interstitial cell	0	na	1	na	1	na	1	na	2	na
Uterus, leiomyosarcoma	na	1	na	3	na	0	na	3	na	4
Urinary bladder, mesenchymal tumor[#]	0	1	0	0	0	0	0	1	0	1

[#]Rare tumors
na- not available

SUMMARY AND CONCLUSIONS:

Adequacy of Studies: Sponsor initiated studies based on saturation of absorption of the parent drug at >400 mg/kg/day prior to review by the Executive CAC on the dose selection. The sponsor, however, did not measure either total radioactivity or metabolites for evidence of saturation of absorption. The ongoing studies were considered acceptable by the Exec CAC based on the unbound AUC ratio of >25-fold compared to a 10 mg therapeutic dose. The AUC ratio for the unbound parent drug of approximately 10 fold in both sexes was not sufficient for the high dose of 400 mg/kg/day for mice at the proposed

clinical dose of 20 mg, which is now the sponsor's proposed human dose. Survival rate (~50% overall) was similar in both control and treated groups at the end of the study without marked changes in body weight, and was sufficient for an adequate assessment of tumorigenic potential. Reduced survival rate in the control group 1 during weeks 56 to 100 compared to the other groups did not appear to affect the overall findings. Major cause of death was urinary tract disorders (inflammation/retention) accounting for 35% in males and lymphoreticular neoplasia in 34% of preterminal females. Neither tumor latency nor historical control data from the laboratory were provided in this study.

Non-neoplastic findings: Sponsor considered the increased incidence of the findings was attributed to normal biological variation and no toxicological significance. Gross lesion of soft testes was associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia with slight increase from 60 mg/kg. The incidence was often associated histologically in euthanized animals with atrophy of the testicular tubular epithelium. Aspermia in the epididymides was considered as secondary to the atrophy. Macroscopic/microscopic lesions in the cecum/rectum and eye occurred at ≥2 fold in the high dose group compared to controls.

Neoplastic findings: IC351 administration for 2 years caused slight increase in the incidence of hemangiosarcomas in multiple tissues at all doses, lung alveolar/bronchiolar carcinoma or combined adenomas/carcinomas of the mid- and high dose females, hepatocellular adenoma/carcinomas of high dose males, and uterine leiomyosarcoma at high dose. These tumors were not statisticlly significant for the Peto's survival-adjusted one-sided trend test analyzed by Proc Multtest. Marginal statistical difference for the combined adenoma/carcinoma in the lung from control group 2 in the mid-dose ($p=0.0053$) and high-dose ($p=0.0099$) with the pairwise test ($p \leq 0.01$ for common tumors) is not likely to be biologically significant since there was no statistical significance compared to control 1 or combined control group. The hepatocellular tumors were not considered to be biologically significant since both benign and malignant tumors were seen concurrently without significant increase in the number of multiple tumor-bearing animals, and the incidences were not associated with differential cell focus/hyperplasia or preterminal deaths. Other rarely occurring neoplasms included malignant meningioma in the brain/optic nerve/pituitary, islet cell adenoma in the pancreas, squamous cell carcinoma in the stomach and malignant luteoma in the ovary found sporadically (1/50) at high dose females.

Study title: 2-Year Oral Gavage Carcinogenicity Study of IC351 in ——— Wistar Rats

Key study findings: There was a non-significant increase in mammary gland adenocarcinomas in mid dose females, uterine adenocarcinomas at high dose females, and hepatocellular adenomas/carcinomas at high dose males. The AUCs for the unbound parent drug were approximately 14 times in males and 26 times in females the human AUC at the proposed clinical dose of 20 mg.

Study number: — '88203 , —'88779 for —— **Volume #, and page #:** vol. 34
Conducting laboratory and location:
Date of study initiation: November 11, 1997
GLP compliance: yes **QA report:** yes (x) no ( )
Drug: IC351 (LY450190, ———
Lot # (% purity): F96/048A (47.0%), F96/038A (45.5%), M95/118A (47.3%), F96/047A (47.8%), F96/046A (46.5%), 43582 (47.1%)
CAC concurrence: Dose selection was not reviewed by the Executive CAC but the committee concurred on the ongoing carcinogenicity studies on 6/16/99 based on AUC ratios with a 10 mg human dose (see Appendix II for report).

Study Type: 2-year rodent bioassay
Species/strain: ——— Wistar rats (*Rattus norvegicus*) **Number/sex/group:** 50/sex/group
Age at start of study: 6 weeks (133-196 g for males and 100-151 g for females)
Animal housing: Individual

Formulation/vehicle: 0.5% hydroxypropyl methylcellulose (HPMC) containing 1% Tween 80
Drug purity/stability/homogeneity: Accessed
Methods:
Doses: 0, 10, 60 & 400 mg/kg
Basis of dose selection: AUC ratios
Route of administration: Oral gavage
Frequency of drug administration: Daily
Dual controls employed: Dual identical controls
Interim sacrifices:
Satellite PK or special study group(s): 3/sex/timepoint for PK
eviations from original study protocol: N/A
Statistical methods: Proc Multtest implemented with the Peto's survival-adjusted one-sided trend test
Observations and times:

Observations	Times
Mortality/Clinical Signs	Twice daily/Detailed physical exam weekly
Body Weights/Food Intake	Weekly
Food Consumption	Weekly/Monthly after 13 weeks
Ophthalmology	Weeks –1, 52 & 104
Pathology/Clinical Chemistry	Week 104
Toxicokinetics	Days 21, 84 & 180 at 0, 0.5, 1, 2, 4, 8, 16 & 24 hrs post-dose

RESULTS: Mortality rate was 14 to 34% for males and 34 to 46% for females at week 104 (see Appendix III for graphical presentation). Clinical signs of fur staining, skin scabbing/reddening and swollen hindlimbs were noted in addition to decreased activity, labored breathing, dehydration, abnormal gait, hunched posture, weakness, uncoordination and mass (located in the urogenital region) seen in dead animals. There were no treatment-related differences in group mean body weight or food consumption between groups, and occasional statistically significant differences seen early in the study were limited to females, and were not considered to be of biological significance (see Appendix IV for graphical presentation). Plasma exposure with T_{max} values of 2 to 8 hrs, increased less than proportionally to the increase in dose with higher exposure in females. Increase in AUC values was observed on Days 84 and 180, suggesting accumulation of IC351 in the plasma on repeated dosing.

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Mortality, week 104	7	20	11	17	17	23	16	24	17	21
Clinical Signs,										
Fur staining, dorsal/ventral aspect	17	12	12	16	17	11	20	18	11	24
Fur thin cover, dorsal aspect	4	15	6	21	12	24	10	21	13	33
Skin scabbing, limbs/paws	25	10	21	11	19	7	23	9	19	17
dorsal aspect	9	5	9	6	5	6	11	6	10	11
Abnormal breathing, labored/shallow	3	3	6	4	11	5	7	5	14	1
Abnormal feces, decreased/absent	7	5	5	7	9	11	6	9	3	8
Vaginal discharge	na	2	na	3	na	2	na	3	na	7
Body Weights	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Food Consumption	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Hematology (%), week 105/106										
Neutrophil segment	37.9	43.2	39.7	42.2	42.6	38.5	39.6	38.8	32.2	38.5
Lymphocytes	58.4	52.9	54.4	54.9	53.8	59.1	57.3	58.4	64.6	58.9
Monocytes	2.0	0.9	2.1	1.1	1.4	1.3	1.6	0.8	1.8	0.8
Eosinophils	1.5	3.0	1.3	1.8	2.2	1.1	1.5	2.0	1.3	1.7
Ophthalmoscopy	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Gross pathology	See below		See below		See below		See below		See below	
Toxicokinetics, 3/sex/timepoint										
$AUC_{0\text{-}24hr}$ (ng•hr/mL) Day 21					15040	25012	32822	66695	62821	121462
Day 84					18498	37667	47246	101442	101843	188675
Day 180					16070	35899	38604	91106	78863	152863
C_{max} (ng/mL) Day 21					1398	1995	2626	3834	4856	6678
Day 84					1604	2612	3249	6458	6821	10079

	Day 180			1199	2709	2315	6600	4543	8225
T_{max} (hr)	Day 21			2.0	8.0	8.0	2.0	2.0	8.0
	Day 84			2.0	8.0	8.0	8.0	8.0	4.0
	Day 180			2.0	4.0	8.0	8.0	4.0	8.0

UR- unremarkable
na- not available
Human AUC_{0-24} at steady state= 7,700 ng•hr/mL at 20 mg/day

Non-neoplastic findings: Sponsor considered all the incidences observed in the present study to be spontaneous/incidental or age-related (See Appendix V for incidence of histopathological findings). Dose-dependent multifocal acute hemorrhages in the stomach were observed in the superficial portion of the mucosa of the glandular portion at all groups. Congestion/edema in the lungs increased dose-dependently in females. Higher incidence of thymus cyst in females was associated with thymic atrophy/involution. Dose-dependent increase in hypertrophy and vacuolation of follicular cells in the thyroid was characterized by enlarged epithelial cells with an abundant vacuolated cytoplasm in all female groups. Urinary bladder dilatation/inflammation was observed with increased incidence in the mid- to high dose group males. Increased hematopoiesis in the bone marrow and extramedullary hematopoiesis in the spleen was secondary to the inflammatory or hemorrhagic findings in various organs. Chronic progressive nephropathy was characterized by thickening of glomerular/tubular basement membrane, tubular epithelial basophilia, dilated tubules, interstitial fibrosis and mononuclear cell infiltration. Compression and ventricular dilatation in the brain noted in all groups were secondary to the tumors of the brain or pituitary. Adrenal hypertrophy and pituitary cyst were observed at all doses with increased frequency. Gross lesions of ulceration/scab were correlated with dermatitis. Table below summarizes the microscopic findings with increased incidence.

Non-neoplastic Findings in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, accessory cortical tissue	0	0	0	0	0	0	0	1	0	1
hyperplasia, cortical focal	10	9	1	9	2	8	6	8	7	9
hypertrophy, cortical focal	3	4	3	5	7	9	7	10	8	8
hyperplasia, medullary	0	0	3	2	4	1	3	1	2	1
Eye, degeneration, lens	3	5	3	3	5	4	6	4	6	7
hemorrhage	0	0	0	0	0	0	0	1	0	1
Lung, congestion/edema	1	1	3	3	2	4	2	5	2	7
inflammation, bronchioalveolar	1	0	0	0	0	0	2	0	2	1
Liver, vacuolation, hepatocellular	25	6	17	11	17	8	12	13	20	13
hepatocytic karyomegaly	0	0	0	0	0	0	0	1	0	1
Lymph node, mandibular, congestion	0	0	0	0	0	1	1	2	1	0
mesenteric, dilatation, sinusal	0	0	0	0	1	0	0	0	2	0
pigment deposits	0	0	0	0	0	0	0	1	0	1
hyperplasia, lymphoid	0	0	1	0	0	2	0	0	2	0
Nerve, optic, atrophy	0	0	2	0	0	2	1	0	1	3
Ovary, cyst	na	4	na	8	na	6	na	11	na	9
degeneration, corpora lutea	na	0	na	0	na	0	na	1	na	1
Pituitary, cyst	9	3	9	4	12	3	15	3	19	8
proliferation, tubular	0	1	0	0	0	0	1	0	2	0
Rectum, hyperplasia, mucosal	0	0	0	0	0	0	2	1	1	0
hemorrhage	0	1	1	0	0	0	1	1	1	0
Seminal vesicle, hyperplasia	0	na	1	na	0	na	0	na	2	na
inflammation	0	na	5	na	3	na	4	na	4	na
Skin, dermatitis	0	0	0	0	0	0	0	0	1	1
folliculitis	0	0	0	0	1	1	0	1	0	1
Spleen, cyst, capsular	0	0	0	0	0	0	0	0	1	1

hematopoiesis, extramedullary	5	10	9	8	10	7	12	7	16	11
hyperplasia, lymphoid	0	0	0	2	1	0	1	0	1	0
Stomach, hemorrhage	4	2	6	3	8	9	8	6	10	6
hyperplasia, epithelial	0	0	0	0	1	0	1	0	1	0
ulceration, glandular mucosa	0	1	0	0	0	1	3	1	1	0
Testis, edema	3	na	0	na	1	na	2	na	2	na
polyarteritis nodosa	0	na	0	na	2	na	1	na	2	na
hemorrhage	0	na	0	na	0	na	1	na	1	na
Thymus, cyst	0	9	4	13	2	18	3	21	0	18
Thyroid, dilatation, follicle	0	0	0	1	1	0	1	0	1	0
hemorrhage	0	0	0	0	1	0	1	0	1	0
hyperplasia, follicular cell	3	1	4	0	3	1	4	1	2	3
hypertrophy/vacuolation, follicular cell	3	1	1	2	1	3	7	4	1	6
Urinary bladder, dilatation	0	0	0	1	0	0	5	0	4	0
inflammation	3	2	2	0	6	1	2	2	6	0

UR- unremarkable
na- not available

Neoplastic findings: There was no statistical significance in the incidence of any tumors compared to each control group with either trend or pairwise test.

Increased incidence of hepatocellular adenomas or combined adenomas/carcinomas was sobserved in the high dose male group.

Neoplastic Findings in Liver in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adenoma, hepatocellular	2	0	4	1	3	5	3	4	6	2
Carcinoma[#], hepatocellular	0	0	0	0	0	0	1	1	1	0
Adenomas+Carcinomas	2	0	4	1	3	5	4	5	7[a]	2

[#]Rare tumors
[a]p=0.1015 compared to control 1 with pairwise test

Mammary gland adenocarcinoma was associated with mass in the subcutaneous tissue. The tumors were observed in all treated female groups including controls with significantly higher incidence in the mid-dose group, but not dose-related. Pairwise comparison showed statistical significance at p=0.01. Sponsor interpreted increased incidence of mammary gland adenocarcinoma in females to be a chance variation and of no biological significance since the incidence ranged within 0-22% reported in the literature.

Neoplastic Findings in the Mammary Gland in — Wistar Rats

Dose, mg/kg	**0**		**0**		**10**		**60**		**400**	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adenocarcinoma	0	1	0	3	0	2	0	10*	0	3

*Statistically significant at p=0.0012 from control 2 with pairwise test

Incidence of uterine adenocarcinomas (10%) was not statistically significant, and considered to be of no biological significance compared to the range of industrial in-house facilities (0 to 20%) for Wistar rats.

Neoplastic Findings in the Uterus in Female — Wistar Rats

Dose, mg/kg, n=50	**0**	**0**	**10**	**60**	**400**
Adenocarcinoma	3	0	1	1	5[a]
Squamous cell carcinoma[#]	0	0	0	0	1
Stromal, polyp	2	4	5	7	6

[#]Rare tumors

*p=0.0208 compared to control 2 with pairwise test

Other rare tumors occurred with IC351 included sarcomas in the colon/pancreas/seminal vesicle, carcinoma in the duodenum, leiomyosarcoma in the jejunum, fibroma/malignant schwannoma in the subcutaneous tissue, and squamous cell papilloma in the stomach observed infrequently (1/50) in the high dose group.

Incidence of Other Neoplastic Lesions in ——— Wistar Rats

Dose, mg/kg	0		0		10		60		400	
	50M	50F	50M	50F	50M	50F	50M	50F	50M	50F
Adrenal, cortical adenoma	0	1	1	2	0	1	0	0	2	1
benign pheochromocytoma	2	0	1	1	0	0	1	1	2	0
malignant pheochromocytoma	0	0	1	0	1	0	2	0	1	0
Colon, sarcoma#, metastasis	0	0	0	0	0	0	0	0	1	0
Duodenum, carcinoma# metastasis	0	0	0	0	0	0	0	0	0	1
Jejunum, leiomyosarcoma#	0	0	1	0	0	0	0	0	0	1
Hemolymphoreticular tissue, malignant lymphoma	0	0	4	0	0	1	4	0	3	1
Lung, carcinoma#, metastasis	0	1	0	0	0	1	1	0	1	1
Lymph node, mesenteric hemangioma	4	0	0	0	1	0	0	0	0	1
Pancreas, adenoma, islet cell	2	0	5	0	7	0	1	2	3	1
sarcoma#	0	0	0	0	0	0	0	0	1	0
Seminal vesicle, sarcoma#, metastasis	0	na	0	na	0	na	0	na	1	na
Skin, adenoma, basal cell#	0	0	0	0	0	0	2	0	0	1
carcinoma, squamous cell	0	0	2	0	3	0	3	0	0	1
Stomach, papilloma, squamous cell#	0	0	0	0	0	0	0	0	1	0
Subcutaneous tissue, fibroma#	0	0	1	0	1	0	0	0	1	0
malignant schwannoma#	0	0	1	1	0	0	0	0	1	0
Thymus, benign thymoma	4	1	1	1	0	3	1	0	2	4
Thyroid, adenoma, C-cell	7	3	2	6	1	0	6	6	3	3
adenoma, follicular cell	4	3	1	0	1	0	4	1	5	1
carcinoma, C-cell	0	2	0	1	0	0	0	0	1	0
carcinoma, follicular cell	0	1	0	1	1	1	0	0	1	0

#Rare tumors
na- not available

SUMMARY AND CONCLUSIONS:

Adequacy of studies: Sponsor initiated studies based on saturation of absorption of the parent drug at >400 mg/kg/day prior to review by the Executive CAC on the dose selection. Sponsor, however, did not measure either total radioactivity or metabolites for evidence of saturation of absorption. Later ongoing studies were considered acceptible by the Exec CAC based on the unbound AUC ratio of >25-fold compared to a 10 mg therapeutic dose. The AUC ratios for the unbound parent drug were approximately 14 fold in males and 26 fold in females the human exposure at the proposed clinical dose of 20 mg. The high dose produced acceptable drug exposure in female rats. Increased mortality in all treated groups from 84- and 48 weeks in males and females, respectively, without significant body weight change, did not appear to affect the overall findings. Neither tumor latency nor historical control data from the laboratory were provided in this study.

Non-neoplastic findings: Sponsor considered all incidences spontaneous, incidental or age-related. However, some incidences occurred at ≥2-fold in the high doses compared to the control groups. These included cortical focal hypertrophy in the adrenal, cyst in the pituitary/thymus, extramedullary hematopoiesis in the spleen, lens degeneration in the eye, stomach hemorrhage, follicular cell hypertrophy/vacuolation in the thyroid, and dilatation/inflammation in the urinary bladder.

Neoplastic finding: The increased incidence of mammary gland adenocarcinoma at mid-dose females, uterine adenocarcinomas at high-dose females, and hepatocellular adenoma or combined adenomas/carcinomas at high-dose males was statistically non-significant and/or within the historical range from the literature. Other rarely occurring tumors included sarcomas in the colon/pancreas/seminal vesicle, carcinoma in the duodenum, subcutaneous fibroma/malignant schwannoma, and squamous cell papilloma in the stomach observed infrequently in the high dose group.

OVERALL INTERPRETATION AND EVALUATION:

Adequacy of the Carcinogenicity Studies and Appropriateness of the Test Model: There was no statistically significant positive trend in survival and statistically significant difference in survival distributions among treatment groups in both mice and rats. The studies were initially conducted based on saturation of absorption at doses >400 mg/kg/day in rats and mice prior to review by the Executive CAC. Later the Exec CAC concurred based on AUC multiples of free drug for rats and mice. Subsequently, the sponsor increased the clinical dose from 10 mg to 20 mg. This resulted in an AUC in humans of approximately 7700 ng•hr/ml (LVDK), nearly 4 times higher than that had been reported for the 10 mg dose. Present studies are not adequate based on PK endpoint since the AUCs for the unbound parent drug below 25 fold in mice (~10 times) for both sexes and male rats (~14 times). It is possible that there is saturation of absorption at doses of ≥400 mg/kg, howerver; parent drug exposure increased slightly with doses up to 2000 mg/kg/day in rats and up to 800-1200 mg/kg/day in mice, and more importantly, there were no data showing that drug metabolites did not accumulate with higher doses.

Evaluation of Non-neoplastic Findings: Sponsor considered the non-neoplastic findings unrelated to treatment with IC351 in both species. Increased incidence of penis protrusion in the high dose mice was consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, accounting for 35% of deaths in males. The incidence was often associated with atrophy of the testicular tubular epithelium in euthanized animals. Gross lesion of soft testes was associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia with increasing incidence at ≥60 mg/kg. The findings at a NOAEL, which were also present in the dog, were equivalent to 2-fold the human unbound AUC exposures. Macroscopic/microscopic findings in the eye increased in high dose mice and rats compared to the controls, and were also found in the 3-month studies.

Evaluation of Major Tumor Findings: The increased incidences of hemangiosarcoma, mammary gland adenocarcinoma, hepatocellular adenoma/carcinoma, alveolar/bronchiolar adenomas/carcinomas, and uterine adenocarcinoma were either not statistically significant and/or within the ranges reported in the literature.

Biological Significance: The numerical increase (2-fold) in hepatocellular adenoma of the high dose males was was not statistically significant, but seemed to be a treatment-related effect. The histopathological findings of hepatic vacuolation and/or necrosis were also found at ≥60 mg/kg/day in the 3-month mice, at 800 mg/kg in the 3-month rat and at ≥45 mg/kg/day in the 1-month dog studies. The NOAEL for liver tumors (60 mg/kg) produced unbound drug exposure levels 5 fold and 6-14 fold greater in mice and rats than in humans taking 20 mg, respectively.

Potential clinical implications of findings: Clinical implications of the liver tumors in the present studies are not conclusive although there was no evidence of genotoxic or statistical carcinogenic potential. IC351 potentiates the effects of NO by increasing intracellular cGMP levels. NO is an important regulator of hepatocyte function (Biochemistry **63:** 766, 1998). Particularly, generation of NO during inflammation is involved in hepatocellular carcinoma (Mutat. Res. **305:** 253, 1994), suggesting NO as a mediator for cancer development during chronic inflammation. If IC351 does have the potential to cause liver cancer, the mechanism involved is not clear. One possibility is the involvement of

NO/cGMP during inflammation. NO levels are elevated in patients with chronic hepatitis/hepatic tumors (Mutat. Res. **305:** 253, 1994), and the iNOS overexpression during hepatic injury and/or cell necrosis may contribute to the carcinogenesis in the liver (Am. J. Physiol. Gastrointest. Liver Physiol. **281:** G626, 2001). Urinary excretion of cGMP was significantly higher in patients with primary hepatoma and preneoplastic liver disease, suggesting the uptake of cGMP by the liver (Acta Med. Okayama **36:**331, 1982). Selenium may modulate the differentiation and proliferation of tumor cells by reducing cGMP levels (Biol. Trace Elem. Res. **15:** 243, 1988). Adverse events of dyspepsia, and increased frequency of cholecystitis, pancreatitis or GI carcinoma (3 of 1173) in IC351-treated patients (ongoing H6D-MC-LVBL study) may represent inflammatory/precancerous conditions in the digestive tract. GI dilation/ GI tract abnormalities were one of the major drug-related effects for the preclinical/clinical studies of sildenafil. There were elevated AST/ALT levels in some patients dosed with IC351 (H6D-EW-LVAZ, H6D-MC-LVBF, H6D-MC-LVCQ). Newly characterized PDE11A is also present abundantly in liver (J. Biol. Chem. **275:** 314, 2000), and investigation of physiological roles of PDE11A may reveal the function of the subtype regulating cGMP in the liver.

Recommendations for further analysis: The 2-year carcinogenicity studies in male rats, and male and female mice were conducted at doses below those recommended by the ICH guidelines (see Exectutive CAC minutes in appendex II) based on the AUC exposures for the 20 mg human dose. The Committee recommended an additional alternative mouse carcinogenicity assay be conducted for Phase IV commitment unless the sponsor provided evidence for saturation of absorption by measuring either total radioactivity or metabolites.

Labeling Recommendations:

VII. REPRODUCTIVE TOXICOLOGY (see Review #3 for IND 54,553)

Embro/Fetal & Postnatal Development including Maternal Function in the Rat (Segment II & III)

Key study findings: A NOAEL of 200 mg/kg/day was determined for F0 maternal toxicity based on reduced body weight gain, and of 1000 mg/kg/day for F0/F1 reproductive toxicity & for F2 developmental toxicity in rats. A NOAEL for F1 development could not be established due to marked decrease in postnatal survival at all doses from the 1st study, but appeared to be 30 mg/kg in a subsequent study.

Study no.: 353010 & 353016 **Volume #, and page #:** vols. 37/38
Conducting laboratory and location:
Date of study initiation: September 20, 1999 (353010)/April 20, 2000 (353016)
GLP compliance: yes **QA reports:** yes (x) no ( )
Drug/lot #: IC351(LY450190)/#980230 **% purity:** 99.69%
Formulation/vehicle: White powder/10% aqueous acacia

Methods:

Species/strain: Crl:CD®(SD) rats
Doses employed: 0, 60, 200 & 1000 mg/kg/day (353010); 0, 3, 10, 30 & 200 mg/kg/day (353016)
Route of administration: Oral gavage
Study design: F0 females were treated from gestation Day 6 through lactation Day 20.
Number/sex/group: 25/female/group
Parameters and endpoints evaluated: f1 pup developmental evaluations (353010 only), F0 females necropsied on lactation Day 21 or post-mating Day 25, gross necropsies on died or euthanized F1, f2 pups euthanized on PND 7 (353010 only), F1 males for spermatogenesis, F1 females necropsied without mating at the end of mating period (353010 only) & surviving F1 females necropsied on postpartum Day 14 or post-mating Day 25 & F1 males

necropsied —— 353010 only), selected F0/F1/f2 animals for histopathology, TK on gestation Day 19 at 0, 1, 2, 4, 8, 16 & 24 hrs post dosing —— -353016)

Results:

—— 353010:

Body weight gain and food consumption were reduced during gestation Days 6-9/6-20/18-20 in all treated groups, suggesting a test article-related effect. Decrease in food intake during the lactation days 10-14/1-14 was also statistically significant in the mid- to high dose groups.

F0 maternal generation

Dose, mg/kg	**0**	**60**	**200**	**1000**
Mortality, n=25	None	None	None	None
Clinical signs	UR	UR	UR	UR
Body weight change (g), gestation, Days 6-9	14	14	13 (↓7%)	10 (↓28%)
Days 6-20	120	118	115 (↓4%)	109 (↓9%)
Days 18-20	35	30 (↓14%)	27* (↓23%)	28 (↓20%)
Days 0-20	152	147	144 (↓5%)	135 (↓11%)
Food consumption (g/kg/d), gestation, Days 6-9	71	67	64** (↓10%)	61** (↓14%)
Days 6-20	68	67	65* (↓4%)	64** (↓6%)
Days 18-20	66	64	60 (↓9%)	60 (↓9%)
Days 0-20	68	67	65 (↓4%)	64** (↓6%)
lactation, Days 10-14	63	61	54** (↓14%)	54** (↓14%)
Days 1-14	150	147	134** (↓11%)	139 (↓7%)
Pregnancy rate, %	96	96	100	92
total litter loss, lactation Day 1	0	0	0	1
non-gravid	1	1	0	2
Gestation length & parturition	UR	UR	UR	UR
Macroscopic findings	UR	UR	UR	UR
Implantation sites, lactation Day 21	UR	UR	UR	UR

UR-unremarkable

Significantly different from control at p=0.05* or p=0.01**

Pups found dead/missing during the f1 postnatal period (PND 0-21) increased markedly in all treated groups without significant general physical signs and necropsy findings except the presence of milk in the stomach in the treated groups. Postnatal pup survival was significantly reduced during PND 1-4 and birth to PND in all dose groups and below the historical control data range (97.6 & 91.3%, respectively) provided by the sponsor.

f1 litter generation

Dose, mg/kg	**0**	**60**	**200**	**1000**
Mortality (PND 0-21), pups (litters), found dead	5(5)	35(14)	50(15)	40(14)
missing; cannibalized	1	19	36	41
euthanized in extremis	0	0	0	1
Clinical signs	UR	UR	UR	UR
Body weights	UR	UR	UR	UR
PND 0 Litter, pups born/litter, live litter size, % males at birth	UR	UR	UR	UR
Postnatal survival, %/litter, PND 1-PND 4	99.1	91.5**	84.5**	85.8**
birth-PND 4	98.4	87.1**	81.9**	79.5**
Macroscopic findings, Stomach, milk present, found dead litters (pups)	0/5 (0/5)	5/14 (5/35)	2/15 (3/50)	4/14 (4/40)

UR- unremarkable

Some animals were found dead or euthanized *in extremis* in the mid- and high dose groups. Decreased body weights observed in PND 21-28, PND 49-56 and gestation Days 15-18 were not considered to be

test article-related. Sperm evaluation data was within the historical values provided by the sponsor. Microscopic findings were considered to be spontaneous, incidental or normal background changes.

F1 generation postnatal deveolpment

Dose, mg/kg	**0**		**60**		**200**		**1000**	
	24M	24F	24M	24F	25M	25F	22M	21F
Mortality, found dead	0	1[A]	0	0	1[B]	0	0	1[D]
euthanized in extremis	0	0	0	0	1[C]	0	0	2[E]
Clinical signs	UR	UR	UR	UR	UR	UR	UR	UR
Body weights, PND 21-28	40.1	33.4	36.8	32.6	35.8*	30.9	35.7*	30.6
PND 49-56	62.6	29.2	61.2	26.9	62.9	30.1	59.0	23.7*
gestation, Days 15-18	na	40	na	42	na	36	na	29*
Developmental sensory function & behavior[#]	UR	UR	UR	UR	UR	UR	UR	UR
Reproductive performance[@]**, fertility index (%)**	91.3	95.7	91.7	95.8	87.0	88.0	90.5	90.5
Gestation length & parturition	na	UR	na	UR	na	UR	na	UR
Spermatogenic endpoint evaluations[$],								
Sperm counts, left testis, no. sperm/10^6/g	95.5	na	87.6	na	91.5	na	84.5	na
left epididymis, no. sperm/10^6/g	476.1	na	421.0	na	428.4	na	433.7	na
Sperm production rate, no. sperm/10^6/g	15.7	na	14.4	na	15.0	na	13.8	na
Sperm morphology, head absent/normal flagellum, %	0.0	na	0.1	na	0.0	na	0.4	na
Implantation sites, lactation Day 14	na	UR	na	UR	na	UR	na	UR
Organ weights, females- fail to deliver, uterus/CX/OD (g)	na	na	na	0.90 (n=1)	na	1.11 (n=2)	na	0.69 (n=2)
Macroscopic findings,								
Liver, white area, lactation Day 14	na	0/21	na	0/23	na	0/22	na	1/17
Urinary bladder/ureter[W], scheduled necropsy	0/24	na	0/24	na	0/23	na	1/22	na
Microscopic findings,								
Kidneys, inflammation, chronic active, moderate	0/2	na	na	na	0/1	na	1/2	na
hyperplasia, transitional cell, mild	0/2	na	na	na	0/1	na	1/2	na
Prostate, inflammation, chronic active, moderate	0/24	na	na	na	na	na	1/22	na

UR-unremarkable
na- not available
Died on Week 19[A], during Week 12[B] or during Week 21[D] (postpartem Day 10)
[C]Euthanized *extremis* during Week 14 due to impaired mobility, labored respiration & swayed while walked.
[E]Euthanized *extremis* in one female during Week 8 (PND 35) due to dehydrated/lethargic, labored respiration & moderate to severe matting on various areas on the body or in the other during Week 9 (gestation Day 22) due to unkempt appearance, decreased defecation, dried red staining on the mouth/nose/forelimbs, & dehydrated, which was diagnosed to be dystocic.
[#]Pinnal detachment, surface righting response, eye opening, balanopreputial separation, vaginal patency, auditory startle test, motor activity & Biel maze swimming trials for learning/memory ability were tested.
[@]Fertility, mating index & estrous cycle were tested.
[$]Testicular/epididymal sperm numbers, sperm repoduction rate, sperm mobility & % of morphologically normal sperm were tested.
[W]Urinary bladder distended/calculi/thickened/reddened mucosa/red fluid contents or ureter distended
Significantly different from control at p=0.05* or p=0.01**

f2 litter generation; There were no remarkable effects of IC351 on the mortality, clinical signs, body weights, PND 0 litter data, postnatal survival & macroscopic findings at necropsy (PND 7) in f2 generation.

— **353016:**

F0 maternal generation; Oral administration of IC351 had no adverse effects on F0 survival, body weights/food consumption, gestation, parturition/maternal function during lactation & macroscopic findings at 3, 10, 30 & 200 mg/kg/day. Plasma exposure was less proportional to the increase in dose, which was consistent with previous studies in the pregnant and non-pregnant rats. Higher exposure was observed in the 200 mg/kg/day group on gestation Day 19 in the present study than on gestation Day 12

in the previous study ($AUC_{1\text{-}24h}$= 63554.5 ng•hr/mL, # —— -353005). Sponsor considered that the different gestation day may affect the exposure to IC351.

Dose, mg/kg, n=23-25	**0**	**3**	**10**	**30**	**200**
Body weight changes, lactation, Days 1-21	32	30	34	38	47*
Toxicokinetics, $AUC_{0\text{-}24h}$ (ng•hr/mL)		10614	31686	55590	91115
C_{max} (ng/mL)		912	1872	3490	6584
T_{max} (hr)		4	4	8	8

*Significantly different from control at p=0.05

f1 generation: Mean postnatal survival rate for the birth to PND 4 in the 30 mg/kg group was below the sponsor's contol data of 91.3% due to the deaths of 7 pups from 1 litter during this interval according to the sponsor.

Dose, mg/kg	**0**	**3**	**10**	**30**	**200**
	23M 23F	23M 24F	24M 24F	25M 24F	24M 24F
Mortality, pups (litters), found dead	12(7)	13(8)	13(8)	27(9)	12(8)
euthanized in extremis	0	0	0	4	0
missing; cannibalized	3	3	3	12	6
Clinical signs	UR	UR	UR	UR	UR
Body weights	UR	UR	UR	UR	UR
PND 0 Litter#	UR	UR	UR	UR	UR
Postnatal survival, birth-PND 4 (%/litter)	96.1	96.4	96.6	89.8	96.5
Macroscopic findings, litters					
Lungs, reddened	0/22	1/24	1/24	3/23	2/24

UR-unremarkable

#Pups born/litter, live litter size & % males at birth were studied.

Summary: —— **-353010;** F1 generation was likely to be exposed *in utero* since parent and/or metabolites of IC351 were detected in maternal placenta and fetal tissues (Study #003R00). No mortality was observed in F0 maternal & f2 generations, but pups or litters found dead increased markedly at all doses during f1 postnatal period (PND 0-21) with one pup in the high dose euthanized *in extremis* without general clinical signs except the presence of milk in the stomach in some of the treated groups. Some rats in F1 generation in the mid- and high dose groups were found dead or euthanized *in extremis*. Sponsor considered the effects not treatment-related since no deaths occurred in the high dose males. Dose-dependent reduction in body weight gain and food consumption for F0 were observed during gestation Days 6-9, 6-20 & 18-20 in all treated groups, suggesting a test article-related effect. Decreased food consumption during lactation days 10-14/1-14 was statistically significant in the mid- to high dose groups, which was attributed to a reduced maternal nutritional demand caused by an increase in pup deaths during PND 1-4 as explained. Reduced F1 mean body weight also occurred sporadically in the mid- to high dose group, which was considered to be unrelated to treatment. Drug exposure via milk is likely to be minimal since maternal milk is not a major route of elimination (≤0.1% in the rat) for IC351 and/or its metabolites (Study #002R00). One male in the high dose group exhibited a distended ureter, dilated renal pelvis, a distended/thickened urinary bladder with calculi/reddened mucosa, chronic inflammation/hyperplasia in the kidney at a scheduled necropsy, and considered to be spontaneous. Postnatal pup survival was significantly reduced during PND 1-4 and birth to PND 4 in all dose groups, and below the sponsor's historical control data. There were no TK data for the study. —— **353016;** Another postnatal growth/survival and TK study at dose levels of 3, 10, 30 & 200 mg/kg/day were conducted to determine the reproducibility and a NOEL. The reduced f1 postnatal survival was not replicated except in the 30 mg/kg/day with a survival rate of 89.8% slightly below the sponsor's historical control data of 91.3%. Sponsor considered a NOEL for F1 developmental toxicity as 30 mg/kg/day.

Conclusion: Administration of IC351 to F0 dams did not affect F1 male/female reproductive performance and fertility. The NOAEL for F0 maternal toxicity was selected at 200 mg/kg/day based on the reduced body weight change/food consumption during gestation. A NOAEL for F0/F1 reproductive toxicity & for F2 developmental toxicity was identified as 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to statistically significant decrease in postnatal survival at all dose groups. In a subsequent study (——→ 353016), postnatal survival was not greatly affected at doses of 3, 10, 30 & 200 mg/kg, and the sponsor suggested the NOEL for F1 developmental toxicity was 30 mg/kg/day. The discrepancies of the findings were not clear since both studies were conducted the same way except for dose selection.

Reproductive toxicology summary: Developmental and reproductivity studies were conducted in rats & mice (refer to Reviews #2 & 3 ———————— Mice were selected as a second species for the embryotoxicity studies due to the poor plasma exposure in rabbits (Study #B00199). Although the drug was not directly administered to the F1 neonates, it is likely they were exposed since IC351 crosses the placenta (Study #003R00). Administration of IC351 had no adverse effects on fertility or reproductive toxicity at doses up to 400 mg/kg/day in either male or female rats. A NOAEL for maternal toxicity (based on the reduced body weight gain) was determined to be 200 mg/kg/day and for embryo/fetal developmental toxicity to be 1000 mg/kg/day in rats. Fetal skeletal malformations/variations in all groups including controls in both rats and mice were within the provided historical data. No other developmental malfunction was noted. A NOAEL for both maternal and developmental toxicity was established as 1000 mg/kg/day in mice. Present studies of combined perinatal/postnatal development indicated that oral administration of IC351 did not adversely affect F0 gestation or parturition up to 1000 mg/kg/day, but significantly reduced the postnatal survival at all doses studied. The cause of death was not determined. Developmental sensory function/ behavior, reproductive performance, implantation sites, spermatogenic endpoints, and mean organ weights in the F1 pups were not markedly affected by maternal treatment up to 1000 mg/kg/day. The number of f2 pups born per litter and postnatal pup survival were also not affected by F0 maternal treatment. The sponsor suggested a NOEL for f1 developmental toxicity to be 30 mg/kg/day. This represents a 9-fold exposure multiple for unbound parent drug compared to the human exposure at a 20 mg dose.

Reproductive toxicology conclusions: There were no significant differences in fertility/mating indices, sperm counts, motility and morphology in IC351-treated male rats up to 400 mg/kg/day. However, Increased incidence of non-reversible testicular seminiferous atrophy/regression and decreased sperm count/aspermia was observed from 10 mg/kg/day in the 3- and 6-month studies and ≥25 mg/kg in the 1-year study for dogs, and 800 mg/kg in the 3-month and ≥60 mg/kg in the 2-year studies for mice. No adverse effects on the estrous cycle, mating, fertility, ovarian weights, numbers of corpora lutea, implantation sites, resorptions and embryos were observed in female rats up to 400 mg/kg. A NOAEL for maternal toxicity in rats was considered to be 200 mg/kg/day based on the decreased body weight gain from both previous and present studies. In mice, a NOAEL of 1000 mg/kg was established for maternal and developmental toxicity since all findings were within the provided historical control data. F1 male and female reproductive performance and fertility were not significantly affected by F0 maternal treatment up to 1000 mg/kg/day. No adverse effects were observed in F1 gestation/parturition/lactation or in f2 pups born per litter/postnatal pup survival. A NOAEL for F0 & F1 reproductive & F2 developmental toxicity in rats was identified as 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to the statistically significant decrease in postnatal survival in all dose groups. In a subsequent study, however, the effect was not observed at 3, 10, 30 & 200 mg/kg, suggesting equivocal toxicological significance.

Labeling recommendations: ————————————

————————————

VIII. SPECIAL TOXICOLOGY

The ocular irritation potential of IC351 was assessed in *in vitro* and *in vivo*, and found to be a mild irritant to the ocular tissue of the rabbit *in vivo*. The *in vivo* acute dermal toxicity was evaluated in rabbits, and determined to be a slight irritant at 1000 mg/kg.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

IC351 was tested in several oral formulations due to the low water solubility and incomplete oral absorption in animals. A ——— of IC351 and was generally used for earlier pharm/tox studies including carcinogenicity studies. A suspension of micronised formulation in 10% acacia for rodents or CMC/SLS for the 1-year dog study, however, did not achieve higher exposure than the ——— Sponsor indicated that dose level was adjusted for adequate exposure depending on the formulation.

IC351 potentiated the relaxant effect of NO in human penile resistance arteries and cavernosal trabecular smooth muscle by inhibiting the hydrolytic inactivation of cGMP by PDE5. It does not increase cGMP levels in cardiac myocytes nor alter the contractile response of papillary muscle in rats. IC351 was 700-fold and its intermediate metabolites were 90- and 250-fold less active against the human photoreceptor PDE6 than against PDE5. IC351 is >10,000-fold more potent for PDE5 than PDE3, an enzyme involved in cardiac contractility. IC351 retains relatively low selectivity for PDE5 vs. human PDE11A (abstract from Am. Coll. Clin. Pharmacol., VA, 2001), which was widely expressed in kidney, liver, pituitary/salivary glands and testis (PNAS 97: 3702, 2000). Thus, pharmacological characterization of IC351 on human PDE11A may provide further clarification of the mechanism of IC351, although the physiological roles of PDE11A are not yet known.

General safety pharmacology studies were conducted in mice, rats, dogs and guinea pigs. Oral administration of IC351 produced a slight-to-moderate ptosis and depression of the pinnal reflex in rats exposed to 200 mg/kg, and vomiting/increased heart beat at ≥10 mg/kg followed by tarchycardia at ≥30 mg/kg in dogs (1/2 at 30 mg/kg and 2/2 at 100 mg/kg). IC351 at oral doses of 1- and 5 mg/kg produced significant, long-lasting reductions in blood pressure in hypertensive rats, and to a lesser extent in normotensive rats. In anesthetized dogs, i.v. administration of IC351 at ≥0.1 mg/kg produced a dose-dependent reduction in blood pressure in the presence of decrease in total peripheral resistance. At oral doses of 20- and 200 mg/kg, decrease in mean arterial blood pressure was observed in conscious dogs without effects on heart rate or respiration rate. The cardiovascular effects, however, were not observed in the repeated dose toxicity studies, partly due to the recording time of 2- to 4 hrs after dosing which is not consistent with maximum plasma concentrations due to high variability (T_{max} ranged from ——— hrs). Significant diuresis and natriuresis were observed in rats at 0.3 mg/kg (i.v.). Repeated daily oral dosing for 21 days produced body weight loss, deteriorated health, bradycardia/heart block, and deaths at 400 mg/kg in conscious guinea pigs. Sponsor considered the ECG changes occurred as a result of the

deteriorated clinical condition of the animals since the effects occurred in the absence of or modification to t and p waves.

Renal clearance is negligible in rats (4 to 6%) and higher in female dogs (25%). Oral bioavailability was 34- to 53% in rats and 10- to 18% in dogs with low plasma clearance, suggesting an incomplete first pass metabolism and the possibility of prolonged absorption. After 24 hrs, the majority of the radioactivity was associated with metabolites in human feces. Exposure increased proportionally over a dose range of 2.5- to 20 mg with a $t_{1/2}$ of 17.5 hours in human. Steady-state plasma concentrations were attained by Day 5 with doses of 10- or 20 mg/day.

The major metabolite in human and dog liver slices is a methylcatechol glucuronide. The same metabolite was present in the rat and mouse in addition to a hydroxyglucuronide and a catechol glucuronide, which was most abundant in the rat. The major glucuronide metabolite in human plasma and urine would be unlikely to have clinically significant effects on any of the human PDEs tested at therapeutic doses based on the known plasma concentrations of the metabolite observed in humans. IC351 was found to be an inducer of CYP1A/CYP2B in mice and rats, and a mechanism-based inactivator of CYP3A in male mice. CYP3A4 was found to be the primary P450 isozyme responsible for the biotransformation of IC351 to the methylcatechol and catechol metabolites.

In rats, the highest concentrations of radioactivity were detected in the GI tract, stomach, adrenals, liver, pancreas, kidneys, lymph nodes, thyroid and lung with greater than plasma concentrations in most tissues after an hour following a single oral dose of 10 mg/kg [^{14}C]-IC351. Twenty four- and 168 hours after dosing, radioactivity was found in the stomach/GI and liver, respectively. The tissue half-life was approximately 10 hours in most tissues except for whole blood (26 hours) and stomach wall (21 hours). There was minimal radioactivity in the CNS in rats. *In vitro* binding of IC351 to human, rat, dog and mouse plasma proteins was determined to be 94%, 92%, 87% and 85%, respectively. Feces was the major route of elimination of radioactivity in humans, rats and dogs, and the majority of radioactivity was excreted in the feces with a recovery of 86% within 72 hours in the rat, indicating incomplete oral absorption and biliary excretion of metabolites.

Daily oral administration of IC351 up to 6 months in mice and rats and up to 1 year in dogs demonstrated that plasma exposure generally increased sub-proportionally to the dose with variable T_{max} of 1 to 24 hours possibly due to variable absorption and saturation of absorption. Plasma AUC values were higher after 1 to 3 months in rats and dogs, suggesting accumulation in the plasma. The AUC and C_{max} values decreased after 1 month in mice, suggesting enzyme induction following multiple dosing. The 6- and 12-month chronic dosing in the dog, however, showed no consistent trends, and there was considerable intra-animal variability in AUC values.

Single dose toxicity was evaluated in mice and rats up to 2,000 mg/kg orally and 100 mg/kg intravenously. IC351 did not cause death at 2,000 mg/kg in mice and rats. The i.v. lethal dose was 100 mg/kg in mice and >62.5 mg/kg in rats. Significant signs of toxicity included labored breathing, jerky movements, subdued behavior, prostration, tremor and convulsions with intravenous doses of ≥37.5 mg/kg in both species. Sponsor considered the majority of clinical signs attributable to the vehicle used (90% PEG).

Maximum tolerated dose toxicity was studied with escalating daily oral doses of spray-dried (0.5% HPMC/1% Tween 80) IC351 up to 2,000 mg/kg/day in rats and up to 800 mg/kg/day in dogs. There were no treatment-related deaths. In rats, clinical signs included reflux of dose, noisy breathing, vocalizing and salivation at 2,000 mg/kg probably due to a large dosing volume. Loose feces (100 mg/kg), vomiting/subdued behavior (200 mg/kg), and pale feces (800 mg/kg) were the major clinical signs observed in dogs with body weight loss at high dose. Thymic atrophy and inflammatory infiltrates in the

stomach/liver were noted. Sponsor considered the maximum repeatable daily doses of 400 mg/kg in rats and 200 mg/kg in dogs with respect to the saturation of plasma absorption.

Repeated dose toxicity was studied for two 3-month studies in mice, 1-, 3-, and 6-month studies in rats, and one 1-, two 6- and one 12-month studies in dogs. In the first 3-month study in CD-1 mice, there was bone marrow hypocellularity, corneal mineralization, liver necrosis, lymphoid atrophy/necrosis in the spleen/thymus at 400 mg/kg given as a ———— with HPMCP. Hepatic vaculation occurred in all treated groups. Daily doses up to 800 mg/kg of IC351 in 10% acacia were administered in the 2nd study. Increased incidence of lymphoid necrosis in the thymus and periarteritis in the testicular arteries was observed at 800 mg/kg. Splenic hematopoiesis was noted in all treated groups. Unilateral hypospermatogenesis and renal tubular inflammation occurred at 800 mg/kg/day.

IC351 was well tolerated in rats at doses up to 400 mg/kg in the 1-, 3- and 6-month studies. Major findings of the 1-month study (— Wistar rats) were thymic periarteritis with hemorrhage foci, perivascular eosinophilic inflammation in the lung, and splenic pigmented macrophage observed in the high dose group given 0.5% HPMC/1% Tween 80 as a vehicle. In the 3-month study (Fisher rats), one male had hepatic necrosis/vacuolation and 1 female had a periarteritis in the mesenteric arteries with multifocal peritonitis at 800 mg/kg given in 10% acacia. Minimal to slight chronic periarteritis characterized by infiltration of the tunica adventitia and media by lymphocytes and monocytes and minimal endothelial hypertrophy and lymphocytic infiltrates in the perivascular connective tissues surrounding the testicular and hepatic arteries were noted with increased incidence at high dose. Histopathologic findings in the 6-month study in — Wistar rats given IC351:HPMCP (———— included brown pigment deposition in hepatocytes/Kupffer cells without splenic hemosiderin deposition at ≥60 mg/kg, and periphlebitis in the mesenteric veins with decreased lymphocytes (males) at all treated groups. Regenerative tubular epithelium/pelvic epithelial hyperplasia in the kidney, hepatic arteritis, hemorrhages in the thymus/lymph nodes and splenic extramedullary hematopoiesis were observed with increased frequency at the high dose.

One high dose male dog (200 mg/kg) in the 1-month study (given in 0.5% HPMC/1% Tween 80) was killed *in extremis* on Day 14 due to ill health secondary to the dissseminated arteritis in the brain, spinal cord, lungs and thymus. This dog showed elevations of neutrophils/total leukocytes/monocytes/ fibrinogen. Other dogs exhibited higher incidence of clinical signs of thin appearance, subdued behavior and loose feces from 45 mg/kg. Statistically significant reduction of body weight gain was observed in the first week at high doses and remained throughout the study. There was an elevation of ST segment indicative of myocardial infarction, pericarditis and myocardial hypoxia in high dose females (2/3). A supplementary examination of the hearts revealed coronary arteritis at ≥45 mg/kg in 1 of each male and female dog. This lesion was not associated with vasodilation/tachycardia but with moderate decrease in heart rate.

A 3-month toxicity study was conducted in male dogs only with IC351:HPMCP ———— at 0, 10, 60 or 200 mg/kg/day in gelatin capsules. Severe bone marrow myelopoisis associated with histiocytic cell infiltration and increased extramedullary hematopoiesis in the liver or spleen were found at the high dose. These dogs also had myocardial degeneration/fibrosis and epicarditis with ulceration/inflammation of the GI tract. Degeneration of the seminiferous epithelium characterized by missing germ cells and/or spermatids was observed in the high dose group during treatment and following recovery. Other histopathologic findings included an accumulation of fine brown pigment in the epithelium of the gallbladder with increased frequency at ≥60 mg/kg.

Two 6-month toxicity studies at oral doses of 0, 10, 60 and 400 mg/kg formulated in IC351:HPMCP ———— were conducted since the first study had arteritis effects considered secondary to Beagle Pain Syndrome (BPS) in the specific colony supplied, and testicular findings due to use of sexually immature

dogs according to the sponsor. High dose dogs (4/6) were killed moribund due to recurrent (≥5) episodes of symptoms of BPS. Clinical signs including thin appearance, subdued behavior, stiff neck and pyrexia developed in 1 mid dose and 6 high dose dogs. Significant body weight loss was noted in the-mid and high dose dogs during the first 12 weeks of the study. Elevated neutrophils/WBC/monocytes/fibrinogen levels and decreased APTT/albumin/calcium levels were seen in the high dose group, consistent with polyarteritis. Increased ALP in the-mid to high doses and decreased RBC/Hb in the high doses were observed and did not return to normal by the end of the recovery period of 1-month. Arteritis was observed in multiple tissues including the spinal cord, thymus, brain, esophagus, urinary bladder, heart (coronary arteries), lungs, ovaries, epididymides, mammary gland and stomach with increased frequency in the high dose group. The pathologist concluded that the effect was either a treatment-related change or exacerbation of spontaneous polyarteritis. Although therapeutic ratios between the exposure for targeted pharmacological activity and the vasculitis could not be determined, a NOAEL of 60 mg/kg produced 3- to 33-fold exposure multiples (due to individual variability) for the unbound parent drug above the human exposure at 20 mg. The second study, however, did not evaluate all target tissues for arteritis. Irreversible testicular atrophy associated with oligo/aspermia was observed with increased frequency at ≥60 mg/kg. The NOAEL gave equivalent exposure for unbound parent drug at a 20 mg clinical dose.

One-year chronic toxicity was conducted in beagle dogs dosed with IC351 in gelatin capsules containing 1% CMC/0.5% SLS at 0, 25, 100 or 400 mg/kg/day. Pale feces were the most frequent clinical signs at all doses. Mean body weight for females in the mid- and high doses was decreased by 6 weeks after the study. Treatment was suspended for 1 of 100- and 1 of 400 mg/kg females between Days 140 and 166, and from Day 196 through the end of the study, due to marked neutropenia with moderate thrombocytopenia. The changes returned to within reference intervals in 2 to 3 weeks after drug removal, but appeared again within 2 weeks after reinitiation of IC351. The 400 mg/kg female had clinical signs of pyrexia, anorexia and lethargy with no vascular lesions but had a single focus of perivasculitis in the circumflex branch of the left coronary artery. Sponsor considered the findings to be idiosyncratic and not a result of a direct toxicity on bone marrow with no inhibition in neutrophil precursors or megakaryocytes. Degeneration and atrophy of the seminiferous epithelium were observed in all treated groups with decreased testicular weight. Aspermia was noted in the epididymides of the most severely affected dogs, and the incidence and severity of the lesions increased following 12 months of dosing. Other histopathologic findings included lymphocytic infiltration in gall bladder and multifocal fibrosis in the prostate observed sporadically at all treated groups. Hepatic leukocytosis/perivasculitis/pigmentation were noted with slightly increased frequency in the high dose group.

Genotoxicity was evaluated in the standard battery of Ames test, a mammalian cell mutation test in mouse lymphoma cells and a cytogenetic test in human peripheral lymphocytes in the presence or absence of metabolic activation as well as World Health Organization (WHO) nitrosation assay *in vitro*. IC351 was also tested in rat bone marrow micronucleus test *in vivo*. IC351 was not cytogenetic, clastogenic or cytotoxic with no evidience of intrinsic genotoxic potential.

Two-year carcinogenicity studies of IC351 given in 0.5% HPMC/1% Tween 80 were initiated by the sponsor in CD-1 mice and — Wistar rats at 0, 10, 60 and 400 mg/kg/day based on saturation of absorption at doses >400 mg/kg/day prior to review by the Executive CAC. The Exec CAC concurred with the doses based on AUC multiples of free drug for rats and mice. Subsequently, the sponsor increased the clinical dose from 10 mg to 20 mg. This resulted in an AUC in humans of approximately 7700 ng.hr/ml (LVDK), nearly 4 times higher than that had been reported for the 10 mg dose. The high dose produced systemic exposure of 10 fold and, 14- and 26 fold in mice and rats, respectively, compared to the human dose of 20 mg, indicating that for mice and male rats adequate exposure was below the 25-fold animal/human ratio recommended by the ICH guidelines. In addition, parent drug exposure increased slightly with doses up to 2000 mg/kg/day in rats and up to 800-1200 mg/kg/day in mice, and

more importantly, there were no data showing that drug metabolites did not accumulate with higher doses.

Major non-neoplastic lesions observed with increased frequency in IC351-treated mice included (1) penis protrusion in the high dose group consistent with inflammation in urinary tract/prostate/seminal vesicle and/or urinary retention, accounting for 35% of male deaths during the study, (2) gross lesion of soft testes associated with atrophy of the testicular tubular epithelium and epididymal oligo/aspermia at ≥60 mg/kg, (3) dark areas/foci in the cecum or edema of the cecum/rectum in the high doses paralleled the amyloidosis in the digestive tract, (4) lens degeneration/corneal mineralization/erosion in the eye at 400 mg/kg and eye opacity at ≥60 mg/kg, and (5) hepatocellular vacuolation correlated with mass/area raised in the liver at at all doses in females.

Major neoplastic findings in mice were hepatocellular adenomas/carcinomas in high dose males, hemangiosarcomas in multiple tissues at all doses and alveolar/bronchiolar adenomas/carcinomas in mid dose females. Mammary gland adenocarcinomas of mid dose females, and hepatocellular adenomas/carcinomas at high dose males were observed with increased frequency in rats. The increased incidence of the tumors was not statistically significant.

Reproductive and developmental toxicity of IC351 given in 10% acacia was assessed in CD-1 mice and SD rats. Mice were used for a second rodent species of embryo/fetal development studies since plasma exposure for rabbits was minimal due to either extensive first pass metabolism or poor absorption. There were no significant differences in fertility/mating indices, sperm parameters (count, motility and morphology) in IC351-treated male rats up to 400 mg/kg/day. However, non-reversible seminiferous tubular atrophy/regression and/or decreased sperm count/aspermia were observed from 10 mg/kg/day in the 3- and 6-month, from 25 mg/kg/day in the 1-year for dogs, and at 800 mg/kg in the 3-month and from 60 mg/kg for the 2-year studies in mice with low multiple exposure (<10-fold) at a 20 mg/day clinical dose. No adverse effects on the estrous cycle, mating, fertility, ovarian weights, numbers of corpora lutea, implantation sites, resorptions and embryos were observed in female rats up to 400 mg/kg. A NOAEL for maternal toxicity in rats was considered to be 200 mg/kg/day based on the decreased body weight gain. A NOAEL of 1000 mg/kg was established for maternal and developmental toxicity in mice. F1 male and female reproductive performance and fertility were not significantly affected by F0 maternal treatment up to 1000 mg/kg/day in rats. No adverse effects were observed in F1 gestation/parturition/ lactation or in f2 pups born per litter/postnatal pup survival. A NOAEL for F0 & F1 reproductive & F2 developmental toxicity in rats was identified at 1000 mg/kg/day. A NOAEL for F1 developmental toxicity in the rat could not be established in the combined segment II/III study due to the statistically significant decrease in postnatal survival in all dose groups. In a subsequent study, however, the same effect was not observed at 3, 10, 10, 30 & 200 mg/kg, suggesting an equivocal toxicological significance. Sponsor considered a NOAEL for a developmental toxicity to be 30 mg/kg/day.

Plasma exposure of IC351 was measured in pregnant mice and rats during gestation and was less than proportional to doses over the range of 60 to 1000 mg/kg, a result also seen in non-pregnant animals. Milk excretion and placental transfer were determined with 10 mg/kg of [^{14}C]-IC351 in pregnant Fischer rats. The radioactivity in maternal milk was less than 0.1%, suggesting that maternal milk is not a major route of elimination and the exposure through maternal milk would be insignificant. IC351 crosses the placenta resulting in fetal tissue exposure, which is substantially lower than maternal exposure.

Special toxicity studies of *in vitro* and *in vivo* ocular irritation or *in vivo* dermal irritation were conducted. IC351 is considered as a mild ocular and dermal irritant in New Zealand White rabbits *in vivo*.

Conclusions: The preclinical toxicology studies conducted with IC351 demonstrate that the drug is generally well-tolerated in mice, rats and dogs.

General Toxicology Issues: Major histopathological findings of IC351 treatment in animals are arteritis and testicular degeneration/atrophy, which were also found in other PDE 5 inhibitors. The testicular findings were observed with increased incidence in mice in the 3-month toxicity study and the carcinogenicity study, and in dogs in the 3-, 6- and 12-month toxicity studies with no/low safety margin compared to the proposed clinical dose of 20 mg. The findings are likely to be irreversible since the incidence was observed during the recovery in the 3- and 6-month dog studies. Sponsor considered that reversibility is unlikely although the reversibility of the finding is unknown for the 1-year study, extensive cell loss in the germinal epithelium was observed in dogs with the most severe testicular alterations. In men, there were no clinically significant effects on semen parameters up to 6 months at 20 mg (#H6D-MC-LVCZ).

Vasodilators such as phosphodiesterase type-III inhibitors are known to cause vasculitis in rats and dogs. In dogs, vasodilators produce a cardiovascular toxicity, which generally includes coronary arteritis and more variably, atrial epicardial hemorrhage and/or focal myocardial necrosis. The arteritis tends to resolve by fibrosis and intimal hypertrophy. A second class of lesions features non-hemorrhagic inflammation, with or without fibrinoid necrosis, of one to three layers of the wall of arterioles and venules, and typically manifested as rash or purpura, although viscera can be involved. The lesions are believed to reflect hypersensitivity, and are the major manifestation of clinical drug-induced vasculitis (Drug-induced vasculitis: interim guidance for industry and FDA pharmacologists Jan. 2002). The relevance to humans and the pathogenic mechanism of drug-related vascular lesions in animals are poorly understood, and the specific biomarkers are not identified. Vasculitis was observed in IC351-treated dogs, mice and rats. For the most part, vascular effects were slight to minimal, not particularly dose-related, and occurred at high doses with exposures to free drug much higher than exposures in men. In rats and mice, IC351 caused minimal to slight vasculitis with moderate multiples of human exposures. In only one instance were there any effects (phlebitis of mesenteric arteries) at doses below the highest dose studied. Drug exposure at high doses were 6-9 times in mice and 7-33 times in rats the human exposure at 20 mg. In dogs, there was perivascular inflammation in the lungs in 3/6 dogs vs. 0/6 in controls at approximately the same exposure as men taking 20 mg in the 1-month study. In a 6-month study, drug-induced exacerbation of Beagle Pain Syndrome associated with both acute- and chronic disseminated arteritis was observed in both control and treated groups with increased incidence and severity at the high dose. However, the lowest free drug concentration in an affected dog was 29 times greater than the free drug concentration in men taking 20 mg. The vasculitis findings were not seen in the 1-year dog study with doses giving exposures up to 33 times the human exposure. Instead, marked thrombocytopenia/neutropenia indicative of type III immunopathy were seen in 1 mid- and 1 high dose female with 14 and 18X the human exposure. The one high dose female also had a single focus of left coronary artery perivasculitis. Hypersensitivity is the major manifestation of clinical drug-induced vasculitis, which is represented by anemia, leukopenia, thrombocytopenia, vasculitis, *etc.* Possible systemic symptoms of hypersensitivity such as myalgia, back pain or infection were the most frequently reported adverse human events associated with IC351.

In dogs, a therapeutic index could not be determined since the exposure for the desired pharmacological activity and the vasculitis was not determined. However, the unbound drug exposure in dogs at a NOAEL was 1- to 3 fold at 10 mg/kg in the 1-month study and 3- to 33 fold at 60 mg/kg/day in the 6-month study (due to individual variability) the exposure of men taking 20 mg. One Phase II clinical study (LVBF) evaluated erythrocyte sedimentation rate (ESR) and serum creatine kinase (CK). There was lack of association found between back pain or myalgia and either increased ESR or serum CK. Sponsor concluded that neither back pain nor myalgia was associated with inflammatory or myopathic etiologies. Additional information from skin biopsies, evaluation of deposition of immune complexes in vessels or plasma/serum elevations in the cytokines/ANCA may be helpful to monitor the hypersensitivity vasculitis.

Recommendations: The preclinical data support the safety of the proposed dose of 20 mg of Cialis.

X. APPENDIX/ATTACHMENTS:

Appendix I: IND 54,553 Reviews 1/2/3

Appendix II: CAC minutes for protocol and final studies
Appendix III: 2-Year carcinogenicity survival rate
Appendix IV: 2-Year carcinogenicity body weight changes versus dose level
2-Year carcinogenicity group body weight summary
2-Year carcinogenicity individual data listing
Appendix V: 2-Year carcinogenicity sponsor's histopathological incidence tables
Appendix VI: Statistical review and evaluation from the agency

Addendum to review: See next page

Any compliance issues:

Addendum to Review

4/12/02
NDA: 21-368
Drug name: Tadalafil
Sponsor: Lilly ICOS LLC
Division: DRUDP, HFD-580
Reviewer: Yangmee Shin

Review of Total Radioactivity for Carcinogenicity Study Doses

Sponsor submitted a summary of the data for saturation of total drug-related substances associated with IC351 by measuring the total radioactivity exposure after oral administration of [^{14}C]-IC351 (specific activity) in male rats, and male and female mice. The animals were administered daily doses of 400, 800 and 1000 mg/kg formulated as the IC351:HPMCP at least for 2 weeks followed by a single dose of [^{14}C]-IC351:HPMCP.

Results:

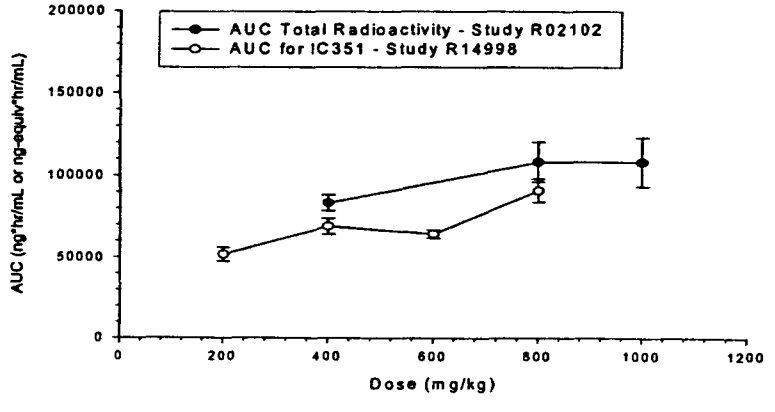
Rats (R02102);
A 2-fold increase in dose from 400- to 800 mg/kg resulted in a 1.3-fold increase in total radioactivity exposure (AUC), and no further increase in exposure at 1000 mg/kg in male rats, suggesting subproportional increases in exposure with increasing dose.

Table 1: Plasma PK Parameters of Total Radioactivity on Day 14 Following 2 Weeks of Daily Oral Administration of 400, 800 or 1000 mg IC351/kg/day to Male Wistar Rats

Parameter	Dose (mg/kg/day) [a]		
	400	800	1000
$AUC_{0\text{-}24hr}$ (ng-equiv*hr/mL)	82973	108068	108034
AUCSEM	4842	12048	14985
C_{max} (ng-equiv/mL)			
T_{max} (hr)	8	4	4

[a] [^{14}C]IC351:HPMCP was administered on the final day of dosing and plasma radioequivalent concentrations were measured after this dose.

Abbreviations: AUC = Area under the plasma concentration time curve between 0 and 24 hours; AUCSEM = standard error estimate of the variability of mean AUC; Cmax = Maximal observed plasma concentration; T_{max} = time to reach maximal plasma concentration.



Mouse;

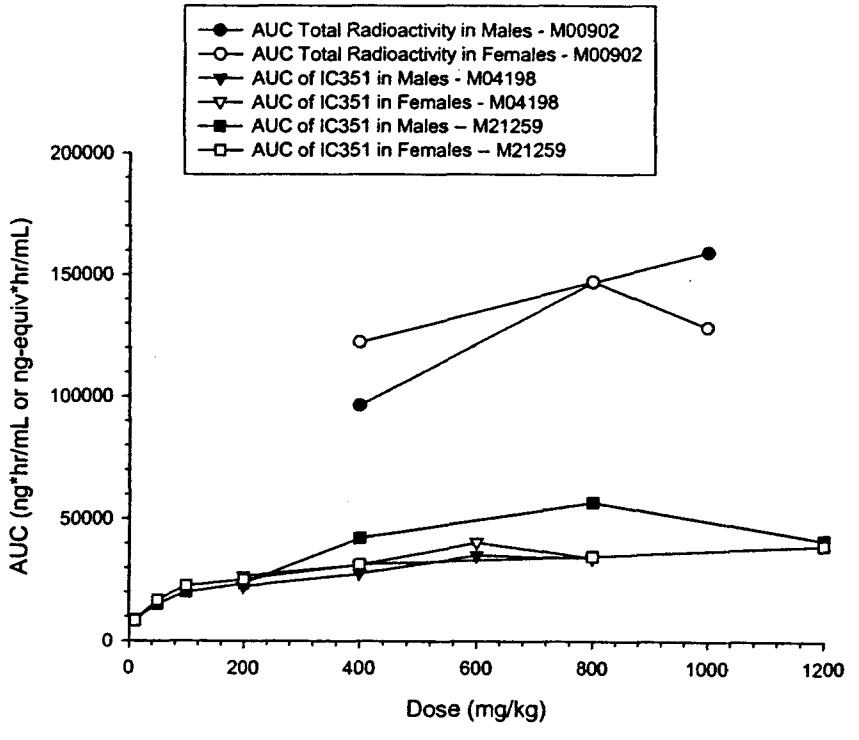
A 2-fold increase in dose from 400- to 800 mg/kg resulted in a 1.5-fold increase in total radioactivity exposure (AUC) in male mice and a 1.2-fold increase in female mice, and 1.1-fold increase in exposure in male mice and 1.1-fold decrease in female mice at 1000 mg/kg. The data suggest that the increase was subproportional to dose. The apparent differences between male and female exposures were considered to be normal study-to-study variability since the radioequivalent concentrations after a single dose (000M02) was different than on Day 16 in the present study. In a pilot PK study in mice with a single dose of 400 mg/kg (000M02), plasma concentration vs. time profile of total radioactivity corresponded to the profile for unchanged IC351, indicating that absorption of IC351 would be expected to govern metabolite disposition. The methylcatechol glucuronide demonstrated formation rate limited PK, as the half-life values were similar for IC351 and the metabolite.

Table 2: Plasma PK Parameters of Total Radioactivity on Day 16 following 2 Weeks of Daily Oral Administration of 400, 800 or 1000 mg IC351/kg/day to CD-1 Mice

Parameter	Dose (mg/kg/day) [a]					
	Male			Female		
	400	800	1000	400	800	1000
Day 16						
$AUC_{0\text{-}24hr}$ (ng-equiv*hr/mL)	96170	146784	159160	122117	147103	128247
C_{max} (ng-equiv/mL)						
T_{max} (hr)	4	6	2	2	2	1

[a] [^{14}C]IC351:HPMCP was administered on the final day of dosing and plasma radioequivalent concentrations were measured after this dose

Abbreviations: AUC = Area under the plasma concentration time curve between 0 and 24 hours; C_{max} = Maximal observed plasma concentration; T_{max} = time to reach maximal plasma concentration.



Conclusion:
In male rats, there was a 30% increase in total radioactivity when the dose was raised from 400 mg/kg to 800 and 1000 mg/kg. In female mice, the increase was 20% at 800 mg/kg and 5% at 1000 mg/kg. Clearly, saturation of absorption was achieved at 400 mg/kg in these two cases. In male mice, doubling the dose from 400 to 800 mg/kg increase total radioactivity 53% and increasing the dose to 1000 mg/kg increased total radioactivity 65%. Although saturation of absorption was not achieved, the increase is clearly non-proportional to dose and is reaching saturation.

Recommendation:
Three of four arms of the two carcinogenicity studies (male and female rats and female mice) are clearly valid. Also, saturation of absorption may have been achieved in male mice since no gender difference in exposure to IC351 has been seen in previous studies in mice and the apparent differences between male and female exposures in the present study may be within the normal study-to-study variability. Furthermore, at 400 mg/kg, the AUC in male mice is 10 times higher than in men taking 20 mg daily. Considering all the data, the carcinogenicity studies for IC351 are acceptable. No additional carcinogenicity studies are necessary.

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/s/

Yangmee Shin
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PHARMACOLOGIST

Alexander W. Jordan
4/29/02 07:39:30 AM
PHARMACOLOGIST

To: Florence Houn
Director ODE III

From: John Leighton
Associate Director for Pharmacology/Toxicology, ODE III

Subject: NDA 21-368
Cialis (tadalafil)

Date: April 23, 2002

Introduction

Lilly ICOS is seeking approval for Cialis for treatment of erectile dysfunction (ED). The sponsor has conducted pharmacology, safety pharmacology and toxicology studies, including 6-month studies in rats and 12 month studies in beagle dogs. Studies to investigate the reproductive toxicity, genotoxicity and carcinogenicity of tadalafil have also been conducted. The Division review of pharmacology and toxicology data submitted by the sponsor support the safety of the proposed 20 mg dose of Cialis

Review of Draft Pharmacology/Toxicology Safety Issues

The Division Pharmacology/Toxicology review noted two outstanding issues to be addressed. These include vasculitis observed toxicology studies in mice, rats and dogs, and a recommendation for an additional alternative mouse carcinogenicity assay for a Phase 4 commitment. This commitment would not be necessary if the sponsor provided additional evidence for saturation of absorption.

The Division Pharmacology/Toxicology proposes addressing the issue of vasculitis observed in animal studies through information in the label. The Division extensively considered the animal findings and their relevance to clinical use of Cialis. I concur with the Division's analysis but recommend that the Division seek the opinion of the Pharmacology/Toxicology Coordinating Committee (PTCC) or appropriate subcommittee to ensure consistent labeling of these findings.

The sponsor provided additional information that demonstrates saturation of absorption at high dose in their carcinogenicity studies. The Executive CAC members participating in the original review concurred with this conclusion. Therefore, the additional alternative mouse carcinogenicity assay is not necessary, and Cialis should be considered negative as tested in the mouse and rat carcinogenicity assays.

No additional pharmacology or toxicology issues remain to be addressed.

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John Leighton
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